TROPHIC DYNAMICS OF PINNIPED POPULATIONS IN ALASKA USING STABLE CARBON AND NITROGEN ISOTOPE RATIOS

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ABSTRACT

Trophic changes in populations of Steller sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*) and harbor seals (*Phoca vitulina*) in the eastern Bering Sea and Gulf of Alaska were studied using stable isotope analysis. Declining populations of all three species of pinnipeds prompted this study to determine if changes in diet, likely resulting from food limitation, contributed to the declines. Stable carbon and nitrogen isotope ratios were analyzed in the vibrissae (whiskers) and body tissues of pinnipeds from 1993-1998 and compared with muscle tissue from prey species during the same time period to determine pinniped trophic dynamics. Vibrissae growth rate studies revealed harbor seal vibrissae are only retained for one year then replaced, while Steller sea lions maintain their vibrissae for several years. Isotopic data from all three species are consistent with diets composed of walleye pollock (*Theragra chalcogramma*) at various times and locations throughout the year. Steller sea lion and northern fur seal vibrissae revealed regular oscillations along their lengths in both carbon and nitrogen isotope ratios that likely corresponded to regional isotopic differences. As these animals moved or migrated from one region to another during the year, they metabolically incorporated the different regional isotope ratios through their prey. Because these animals return to their rookery to pup, breed and molt each year, the isotope ratios in the vibrissae showed a regular pattern of enrichment and depletion. Harbor seals, which tend to stay in one geographic location, have relatively static isotope ratios in their vibrissae,

while seals that moved into offshore waters had fluctuating isotope ratios that corresponded to regional differences. No trophic shifts, as evidenced by major changes in nitrogen isotope ratios, were present in any tissues from the three species over the period 1975-1998. Stable isotope ratios of bone collagen for all three species from 1950-1997 indicated no change in trophic level but did reveal a decline in the carbon isotope ratios. These data are supportive of evidence that the seasonal primary production in the North Pacific Ocean has declined and may have contributed to a decreased carrying capacity impacting these top trophic level organisms.

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No man is an island, entire unto himself.
Every man is a piece of the continent,
a part of the main.

-- John Donne

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CHAPTER 1

INTRODUCTION

The Alaska populations of Steller sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*) and harbor seals (*Phoca vitulina*) have undergone various degrees of decline during the past three decades, particularly in the western Gulf of Alaska and Bering Sea (Pitcher 1990; Loughlin 1993; Lewis 1996; Strick et al. 1997). These pinnipeds are generally found in coastal waters and along the continental shelf throughout the North Pacific Ocean, including the Bering Sea and the Gulf of Alaska (NRC 1996). The Steller sea lion decline was first noted in the Aleutian Islands during the 1960s while northern fur seals have experienced population reductions since the 1950s on the Pribilof Islands (York 1990, Loughlin et al. 1992). Harbor seal populations in the western Gulf of Alaska were observed to be decreasing in numbers as early as 1976 (Pitcher 1990).

Food limitation has been hypothesized as a likely cause behind the declines in the pinniped populations, resulting from decreases in prey populations and emigration of certain species (Merrick et al. 1987; Alverson 1992; Loughlin et al. 1992; Trites 1992; Alaska Sea Grant 1993; Merrick 1995; Anderson et al. 1997; Merrick et al. 1997). During the same time period and in similar regions, piscivorous seabirds have also declined (Piatt and Anderson 1996). These declines have raised questions and concerns regarding not only the health of these pinniped populations but also what indication they may be of the condition of the marine environment. Shifts in

commercial fishery stocks, e.g. pandalid shrimp and clupeids (Anderson et al. 1997), as well as these pinniped declines may be large-scale indicators of substantial changes in productivity of the ecosystem.

Scope of study

The goal of this study was to examine the present trophic levels of harbor seals, Steller sea lions, northern fur seals and their probable prey species to determine if any trophic changes had occurred over time that may have contributed to the population declines. I hypothesized that seals and sea lions made dietary changes as a result of changing prey abundances and the $\delta^{13}C$ and $\delta^{15}N$ values before and after the mid-1970s, when the latest major climatic shift occurred, would reflect the changes in trophic level.

Vibrissae (whiskers) are growing tissue found in all pinniped species and were chosen as the primary tissue for stable isotope analysis as they would represent feeding both spatially and temporally by these animals. By comparing the isotope ratios found along the lengths of vibrissae with the isotope ratios of muscle from suspected prey items, changes in food sources and habitat could be surmised for the temporal span represented by the vibrissae.

Chapter 2 presents the results of growth rate experiments on harbor seal and Steller sea lion vibrissae. To adequately compare the isotope ratios of prey items to those in the vibrissae, growth rates needed to be established for the vibrissae. Growth

measurements were gathered on captive animals and growth data were also acquired for vibrissae from wild, recaptured seals and sea lions.

The trophic dynamics and spatial distribution of Steller sea lions and northern fur seals in the Bering Sea and western Gulf of Alaska are addressed in Chapter 3. Fractionation differences in stable isotope values between pinniped muscle and vibrissae were calculated and the vibrissae isotope values were adjusted for the enrichment so that muscle values (pinniped vs. prey) could be directly compared. These values were compared with probable prey items in ten locations from the central Bering Sea through the Gulf of Alaska and along the North Pacific coast of Canada and the United States.

Chapter 4 addresses the variability of isotope ratios in harbor seals residing in Prince William Sound, Alaska. A food web was constructed of likely prey items within and outside the sound and compared to the vibrissae values. Fractionation differences in stable isotope values between harbor seal muscle and vibrissae were calculated and the vibrissae isotope values were adjusted for the enrichment so that muscle values (seal vs. prey) could be directly compared.

Long-term changes in trophic level and environment are discussed in Chapter 5. Five decades of carbon and nitrogen isotope ratios from Steller sea lions, northern fur seals and harbor seals were analyzed in an effort to determine if a change in trophic level occurred and if it contributed to the population declines.

Chapter 6 summarizes the results of the previous chapters and how they can be utilized in trophic studies of pinnipeds. A general conclusion and discussion of future research are also included.

Pinniped declines

The possible anthropogenic effects that might contribute to the pinniped population decline are numerous and include: commercial over fishing of food sources, increased energy expenditure, entrapment by fishing debris, direct and indirect fishery takes and harvesting, (Alverson 1992; Trites 1992; Trites and Larkin 1996; Alaska Sea Grant 1993; Merrick 1995). Any one or combination of these variables may have contributed to the declines.

Commercial fishing may be reducing the pinniped food resources, which in turn would reduce growth rates, lower productivity and even cause starvation (Alaska Sea Grant 1993). There has been a general decline in forage fish abundance during the similar time period (Alverson 1992; Anderson et al. 1997) that could impact the health of these animals by increasing energy expenditures in search of prey and by changing the nutritional quality of their diets.

Historically, most pinnipeds have been viewed as problematic to the Alaskan fishing industry. Seals and sea lions have become entangled in fishing nets, as well as cast-off monofilament and polypropylene, which then results in repair costs and time to the fishermen and often-times death to the mammals (Mathisen 1959). Steller sea lions, harbor seals and northern fur seals have been competing with fishermen for

commercially important fish since the late 1800's. A bounty system was established on harbor seals and sea lions in 1927 in Alaska and not eliminated until the 1960's when the system was shown to be ineffective (Matkin and Fay 1980). Commercial harvests of sea lion pups and bulls, as well as male northern fur seals, were practiced until terminated by the Marine Mammal Protection Act in 1972 (Interagency Task Force 1978, Scheffer et al. 1984). Beyond the harvesting of these animals, it is unknown to what extent these population declines are related to one another or coincidental.

Steller sea lions

Steller sea lions are a wide-ranging species but spend up to 6 months of each year at a rookery to pup and breed. The rest of the year is spent feeding between various haul-out sites (Kenyon and Rice 1961). Steller sea lions are found around the Pacific Rim from the Kuril Islands through the Sea of Okhotsk and southern Bering seas along the Alaska coast and south to California. Most of the world populations breed at rookeries in the Gulf of Alaska during June and July (Loughlin et al. 1984).

In spite of reduced hunting, surveys conducted from 1973 to 1989 of different Alaskan rookeries and haulouts indicated a decrease in population sizes from 196,000 to 81,000 for Steller sea lions (Loughlin et al. 1984, 1992). Steller sea lion populations initially began to decline in the eastern Aleutians in the early 1960s. Declines in the central Gulf of Alaska were noticed by the late 1970s, and by the early 1980s, declines in the western Gulf of Alaska and central Aleutians were noted. The population in

southeast Alaska has remained relatively stable (Loughlin et al. 1984; Merrick et al. 1997).

Northern fur seals

Northern fur seals travel from the Bering Sea southward in winter into the North Pacific as far south as the California/Mexico border in the eastern Pacific Ocean before returning to the home island for pupping and breeding each year (Kajimura 1984; Kajimura 1985; Goebel et al. 1991; Loughlin 1993; Merrick 1995; NRC 1996). Three-fourths of the world's population of northern fur seals uses the Pribilof Islands as breeding grounds during the summer. These seals often spend half the year at sea (November-May) and the remainder of the year at their home island for breeding and pupping (Sinclair et al. 1994). Northern fur seals feed opportunistically on schools of small fish as they migrate between the Bering Sea and the Gulf of Alaska. Seals in the spring (April to June) have diets consisting primarily of gonatid squid (Perez and Bigg 1996). Little is known about the juvenile seals' diets after weaning (one year) (Trites 1992).

Commercial harvesting has impacted the northern fur seal populations throughout this century. In an effort to increase productivity, females, comprising one-fifth of the population, were commercially culled between 1956 and 1968. The hoped-for increase in production never occurred and the Pribilof Islands population has declined over 50% since its peak in 1950 and has not recovered in spite of a cessation

of commercial hunting in 1985. The decline appears to have leveled off on St. Paul Island in 1995 while it continues on St. George Island (York 1990; NRC 1996).

Harbor seals

Harbor seals are found in the Bristol Bay in the southeastern portion of the Bering Sea and coastally throughout the Gulf of Alaska and as far south as California. The individual seals tend to show strong site affinity and were thought to have home ranges of only a few kilometers. Recent studies by Frost et al. (1999) have shown a number of tagged seals in Prince William Sound, Alaska leaving to feed in nearby coastal locations in the Gulf of Alaska (Pitcher 1980).

Areas in the Gulf of Alaska and Prince William Sound have seen dramatic declines in their harbor seal populations. Limited data indicate population declines of harbor seals have been occurring in the southeastern Bering Sea, western Gulf of Alaska and Prince William Sound since the mid-1970s. Between 1976 and 1988, the population on Tugidak Island in the western Gulf of Alaska declined 85% but appears to now be remaining stable (Pitcher 1990; NRC 1996; Small et al. 1998). The harbor seal population in the eastern and central portion of Prince William Sound declined by 40% between 1984 and 1988. The 1989 oil spill from the *T/V Exxon Valdez* resulted in the death of approximately 300 seals and a 26% reduction in pups that year. A 63% overall population reduction has occurred from 1984-1997 (Frost et al. 1999). Southeast Alaska, however, has maintained relatively stable populations of about

12,000 harbor seals, and they are thought to be increasing slightly (Pitcher 1990; Small et al. 1998).

Prey species

There are four commercial fishing regions in the northeastern Pacific Ocean: the eastern Bering Sea, international waters of the Bering Sea, the Aleutian Island region and the Gulf of Alaska. Walleye pollock (*Theragra chalcogramma*) constitutes three-quarters of the forage fish catch in the Bering Sea and a pollock fishery has developed in the Aleutian Islands and in Prince William Sound. Meanwhile, the Gulf of Alaska fishery has long targeted Pacific herring (*Clupea harengus pallasi*), although catches have diminished since 1971 (OCSEAP 1987; Anderson et al. 1997). Pollock is the most abundant commercial species in the Bering Sea and the Gulf of Alaska and is also important in the diets of other fish, seabirds, and marine mammals. Walleye pollock are the primary food source of fur seals in the Bering Sea and a dominant food source of Steller sea lions but may be less important in the Gulf of Alaska (Kajimura 1984, Springer 1992). Humans have now become the largest consumer of adult pollock and are in direct competition with pinnipeds (Trites 1992).

Many forage fish stocks have dramatically increased while others have declined in the Gulf of Alaska since the mid-1970s. The species composition for the region has shifted from an environment dominated by clupeid fishes and shrimp to one currently dominated by gadids and pleuronectids (Anderson et al. 1997). Stomach samples of both seals and Steller sea lions indicate prey items include herring, capelin, sandlance,

pollock and squid (Lowry 1982). In 1951 and 1964, samples from Steller sea lion stomachs from the Bering Sea showed that walleye pollock was the fourth most prevalent prey species (Fiscus and Baines 1966), but by 1976, pollock was the dominant prey item (Lowry et al. 1989). Stomach content analyses of Pribilof Island fur seals in the early 1980s showed a predominance of juvenile walleye pollock and squid. Pacific herring and capelin, previously considered important prey, were absent (Sinclair et al. 1994). Kenyon (1965) noted harbor seals from Amchitka Island in the Aleutian Archipelago had remains of octopus and Atka mackerel in their stomachs while harbor seals sampled in 1979 from the Alaska Peninsula had primarily walleye pollock and octopus in their stomachs (Pitcher 1980).

The biological responses to environmental changes have manifested themselves in fluctuating phytoplankton abundance, zooplankton production and shifting migration patterns and recruitment of salmon (*Oncorhynchus* spp.), capelin (*Mallotus villosus)* and walleye pollock (*Theragra chalcogramma*) (Venrick et al. 1987; Ebbesmeyer et al. 1991; Brodeur and Ware 1992; Hollowed and Wooster 1992; Francis and Hare 1994; Polovina et al. 1994, 1995; Quinn and Niebauer 1995). Anderson et al. (1997) noted changes in demersal prey communities, consisting of both commercial and non-commercial species, in the western Gulf of Alaska in the late 1970s. These changes are expected to be reflected in upper trophic level organisms that utilize those prey. For top consumers such as seals and sea lions, prey availability may fall below threshold densities necessary to sustain recruitment into the population.

Stable isotope ratios

Shifts in stable isotope ratios, $^{13}C/^{12}C$ and $^{15}N/^{14}N$, are used as natural tracers of food intake and are often used to reconstruct the diet of an organism, as the carbon and nitrogen isotope ratios reflect the food consumed and assimilated. The isotopic ratios of animal tissues, particularly in marine organisms, are slightly more enriched in ^{13}C (0.5 to 1‰) and ^{15}N (3 to 5‰) than those found in the diet (DeNiro and Epstein 1978, 1981; McConnaughey and McRoy 1979; Rau et al. 1983; Fry and Sherr 1984; Minigawa and Wada 1984; Sholto-Douglas et al. 1991; Hobson and Welch 1992; France and Peters 1997). Carbon isotope ratios are incorporated into the proteins of a consumer following digestion in a conservative fashion and reflect differences in plant carbon at the base of food webs and metabolic pathways in the organisms. Nitrogen isotope ratios, in contrast, change with trophic level in a predictable manner and can be used to identify seasonal or geographic changes in trophic level during migrational movements (DeNiro and Epstein 1978, 1981; McConnaughey and McRoy 1979; Hobson and Welch 1992).

Isotopic variations are observed in organisms throughout the marine environment. Stepwise enrichment in carbon and nitrogen isotope ratios occurs with each increasing trophic level and in all tissues. Mostly-herbivorous zooplankton, consisting primarily of calanoid copepods and euphausiids in the North Pacific Ocean, are first- and second-order consumers of primary productivity. These zooplankton are integrators of the stable carbon and nitrogen isotope ratios of phytoplankton that are

then passed through the food web to higher-order consumers. Any changes affecting the stable isotope ratios within the phytoplankton, such as inorganic carbon source and growth rate, would be carried through the food web and be reflected in foraging pinnipeds. Likewise, zooplankton reflect the $\delta^{13}C$ of the primary producers upon which they had recently fed. The ^{13}C and ^{15}N values in fish prey species reflect the ratios of their zooplankton and piscivorous prey. Pinniped tissues, in turn, reflect the integrated isotope ratios of assimilated food items. Each trophic level provides temporal and spatial isotopic data necessary to study food web dynamics of pinnipeds (Tieszen et al. 1983; Mizutani and Wada 1988; Michener and Schell 1994).

CHAPTER 2

VIBRISSAE GROWTH RATES OF HARBOR SEALS (*PHOCA VITULINA*) AND STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

ABSTRACT

Vibrissae, which act as a temporal record of feeding in harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*), had growth rates estimated using ^{13}C- and ^{15}N-labeled glycine followed by stable isotope analysis. The labeled glycine was incorporated into keratin and served as a temporal marker for growth rate calculation. One captive harbor seal received two doses 147 days apart while a second seal received one dose; vibrissae were analyzed after 86 and 154 days. The peak positions indicated growth began in the fall, continued into spring but ceased in June with active growth rates of 0.33 mm/d. Two adult captive Steller sea lions each received two labeled doses during a 308 day-period. After 427 days vibrissae in both sea lions showed two peaks corresponding to the markers; growth rates were calculated as 0.05 - 0.07 mm/d. Growth rates in captive juvenile and wild adult Steller sea lions, 0.10 - 0.17 mm/d, supported the assumption that major isotopic oscillations in vibrissae of wild sea lions were annual. The multi-year records imply Steller sea

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Hirons AC, Schell DM, St. Aubin DJ (in press) Vibrissae growth rates of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Can J Zool

lions retain their vibrissae while harbor seal vibrissae, in contrast, have periods of rapid growth and appear to be shed, at least in part, annually.

INTRODUCTION

Vibrissae (whiskers) are keratinous, hair-like structures but differ considerably from pelage (hair). The structure of vibrissae follicles is similar to that of pelage follicles; however vibrissae are larger overall, highly innervated, with large blood sinuses, and controlled by voluntary muscles. The whiskers on pinnipeds occur in the musculature on the muzzle and above the eyes; most of these muscles control the positioning of the vibrissae (Ling 1977). Dehnhardt and Kaminski (1995) described how the vibrissae of harbor seals are capable of complex movement. The seals could discriminate diameter differences among disks by touching them with their mystacial (muzzle) vibrissae. The vibrissae from harbor seals and Steller sea lions have some anatomical differences from each other. In the otariids, or eared seals, including Steller sea lions, the vibrissae shafts are outwardly smooth and taper from the base to the tip, while those in harbor seals and other phocids have a waved surface and possess a more uniform diameter along their length. Otariids tend to have shorter anterior and longer posterior mystacial vibrissae and phocid vibrissae have a more uniform length. No known information exists regarding the significance of these characteristics in different species but differences in the vibrissae structure may be associated with slightly different functions.

Ling (1966) determined that elephant seal vibrissae were not shed during their annual pelage molt but were shed periodically only after the seals were older than two years of age. However, the marsupial *Tricosurus vulpecula*, as noted by Lyne et al. (1974), had prolonged but variable vibrissae growth cycles compared to its pelage. Pelage in pinnipeds is molted annually and replaced during this time. No additional growth in their fur is observed throughout the year (Scheffer 1962; Ling 1970). Because vibrissae appear to function as sensory structures, periodic replacement or renewal due to physical damage could be selectively more advantageous than total seasonal replacement. Based on this information, we hypothesized that these two species of pinnipeds would maintain their vibrissae from year-to-year and they would grow continually, but the uncertainty of their seasonal growth prompted this study. Understanding the relationship between growth and isotope ratios in the vibrissae will facilitate interpretation of the isotopic data as a temporal record of food consumption by these animals. This paper describes the results of vibrissae growth measurements on captive animals and the interpretation of vibrissae data from wild harbor seals and Steller sea lions as related to a larger study on the impact that trophic changes have on these populations.

Severe declines in harbor seal and Steller sea lion populations have been recorded in the Bering Sea and Gulf of Alaska for more than two decades (Pitcher 1990; Strick et al. 1997). No cause and effect relationships have yet been established; however, the concurrent increase in commercial fishing pressure may be causing a

food competition that has pinnipeds competing for many of the same resources. Food limitation has been hypothesized as the likely cause behind the declines in the pinniped populations, resulting from decreases in prey populations and/or alteration of the prey base (Alaska Sea Grant 1993; Alverson 1991; Anderson et al. 1997; Merrick et al. 1997). Trophic changes resulting from diet switching would be recorded in the stable isotopes ratios in the animals' vibrissae.

Vibrissae are growing tissue found in all pinniped species. Vibrissae from seals and sea lions contain a timeline of stable isotope ratios derived from prey items (this study). By comparing the isotope ratios found along the lengths of vibrissae with the isotope ratios of suspected prey items, changes in food sources and habitat can be surmised for the temporal span represented by the growth of the whisker. As part of this larger study, we attempted to determine how growth rate patterns changed in the vibrissae of harbor seals and Steller sea lions throughout a year in order to interpret the isotopic ratios.

MATERIALS AND METHODS

Dosing Experiment

Two adult male harbor seals and two adult female Steller sea lions were maintained for 2-8 years in an outdoor exhibit at Mystic Aquarium in Connecticut. All were born in captivity; the sea lions were transferred from another facility. They were fed a daily ration of vitamin-supplemented fish (herring, capelin, mackerel, smelt) and

squid at a rate that sustained appropriate body weight. Glycine enriched with ^{15}N or both ^{13}C and ^{15}N isotopes (98%) (Cambridge Isotope Laboratories, Andover, Mass,) was employed to mark vibrissae due to the high mole percentage (8.6%) of glycine found in vibrissae keratin. It was administered intravenously as a solution of 100 mg/ml in sterile physiological saline at a dosage of 5 mg glycine/kg of body weight. All procedures were approved by the Institutional Animal Care and Use Committees of both the Mystic Aquarium and the University of Alaska Fairbanks and were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

Table 2.1 details the sequence of label additions and whisker clipping. The glycine was metabolically incorporated into the keratin during growth of the whiskers. Whole blood samples of 1-2 ml were collected prior to dosing and at twenty-four hour intervals for 48 to 72 hours after dosing to monitor the clearance of the label. Whiskers were allowed to grow for a minimum of five months before a second dose of glycine was administered. The second peak was desired to establish two known dated markers in order to calculate growth rate. After a minimum of five more months, a whisker was cut as close to the skin as possible from each animal and analyzed for stable isotope ratios at close intervals along its length to locate the markers (Table 2.1).

Table 2.1. Chronology of vibrissae growth rate experiment in captive harbor seals and Steller sea lions, 9 January 1996 through 9 July 1998.

	HS-N	HS-P	SSL-L	SSL-S
Glycine label	2 - $^{13}C\&^{15}N$	1 - $^{13}C\&^{15}N$	1 - $^{13}C\&^{15}N$ 1 - ^{15}N	2 - ^{15}N
Delivery date	9 Jan. 1996 4 June 1996	4 June 1996	18 June 1996 22 Apr. 1997	20 Aug. 1996 22 Apr. 1997
Sample date (vibrissae)	29 Aug. 1996 5 Nov. 1996 9 July 1998	5 Nov. 1996 9 July 1998	23 June 1998	17 Nov. 1996 23 June 1998

Day # (relative to Jan. 1, 1996)

	1	100	200	300	400	500	600	700	800	900
HS-N	CN		CN	V	V					V
HS-P			CN		V					V
SSL-L			CN			N				V
SSL-S				N		N				V

(CN = carbon and nitrogen label, N = nitrogen only label, V = vibrissae collected)

Natural abundance

A second type of growth rate study using endogenous markers was conducted simultaneously at the Vancouver Aquarium in British Columbia, Canada, on one male and one female subadult Steller sea lion. Both the animals were born in the wild but were found abandoned as pups, two months or less in age, and taken to the Aquarium. Vibrissae were clipped from the muzzle of each of the animals periodically during a three-year period. The vibrissae were analyzed for the inherent variability in stable isotope ratios in animals fed a weight-sustaining diet (herring and pollock) and all the whiskers from an animal were plotted together along a timeline. Overlap in growth from one vibrissa to the next was measured from an inflection point obvious on at least two separate segments. The date of each clipping was known and the growth rate calculated.

Whisker growth in wild harbor seals and Steller sea lions

An adult male harbor seal in southeastern Alaska was captured in September 1994 by the Alaska Department of Fish and Game, and again 7 months later in April 1995. A vibrissa was removed on each occasion and the patterns in the isotope ratios were compared in an effort to determine the average growth rate during the elapsed time period. In 1997 a second harbor seal was recaptured and a whisker removed for isotope analysis two years after a whisker was initially collected and sampled. A third harbor seal, a yearling, was also recaptured one year after it had been initially sampled as a pup.

One to two vibrissae were sampled from thirty subadult and adult sea lions. These animals were sampled on the Pribilof Islands in the Bering Sea and Chirikof Island in the western Gulf of Alaska by researchers from the National Marine Fisheries Service and the University of Alaska. Their vibrissae were analyzed for carbon and nitrogen isotope ratios and used for comparison to the captive animals. All ten of the sea lions sampled in the Gulf of Alaska were adult females, while the sea lions from the Pribilof Islands in the Bering Sea were almost exclusively male and 65% were less than five years of age.

All the mystacial vibrissae were from an adult female harbor seal harvested at Sitka, Alaska and an adult female Steller sea lion harvested at St. Paul, Pribilof Island, Alaska by Alaska native subsistence hunters. The vibrissae were pulled and analyzed for carbon and nitrogen isotope ratios. The patterns of isotope ratios were compared among each animal's vibrissae, particularly the anterior versus posterior whiskers, to determine if growth rates varied among the seal vibrissae and the sea lion vibrissae.

Laboratory procedures

Vibrissae were scrubbed with steel wool to remove any debris and segmented at 1.5 mm intervals from the base to the tip. Blood samples were dried for several days at 60°C and then ground for homogeneity. Stable isotope ratios were determined using a Europa 20/20 continuous flow isotope ratio mass spectrometer (CF-IRMS). Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation:

$$\delta X\ (‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio respectively. $R_{standard}$ for ^{13}C is Pee Dee Belemnite; for ^{15}N it is atmospheric N_2 (air). Peptone was utilized as a reference check for machine drift for every ten samples. Analytical error for samples was approximately ± 0.1‰ for both carbon and nitrogen.

RESULTS

Following injection of the labeled glycine, isotopic analyses of blood samples in both captive seals and sea lions showed a rapid increase in both $\delta^{13}C$ and $\delta^{15}N$. The nitrogen isotope ratio changes were the most pronounced, reflecting the higher ratio of label to ^{15}N in the body composition. Decreases in the blood $\delta^{13}C$ and $\delta^{15}N$ over time indicated loss of the isotope through respiration (carbon) or excretion and incorporation into body proteins (DeNiro and Epstein 1978, 1981).

Harbor seals

The vibrissae of the first harbor seal sampled in August 1996, HS-N, showed only one peak despite receiving two doses of labeled glycine 84 and 233 days earlier. An identical peak was in approximately the same location in a vibrissa collected after an additional 68 days (November 1996) (Figure 2.1). The second harbor seal (HS-P) had the label administered in June 1996 and had a whisker cut in November after 155 days. No marker was evident in HS-P's whisker (Figure 2.2) in spite of high concentrations of the label in the blood samples confirming the availability of the labeled amino acid (Figure 2.3). No marker was evident in either seal from the time

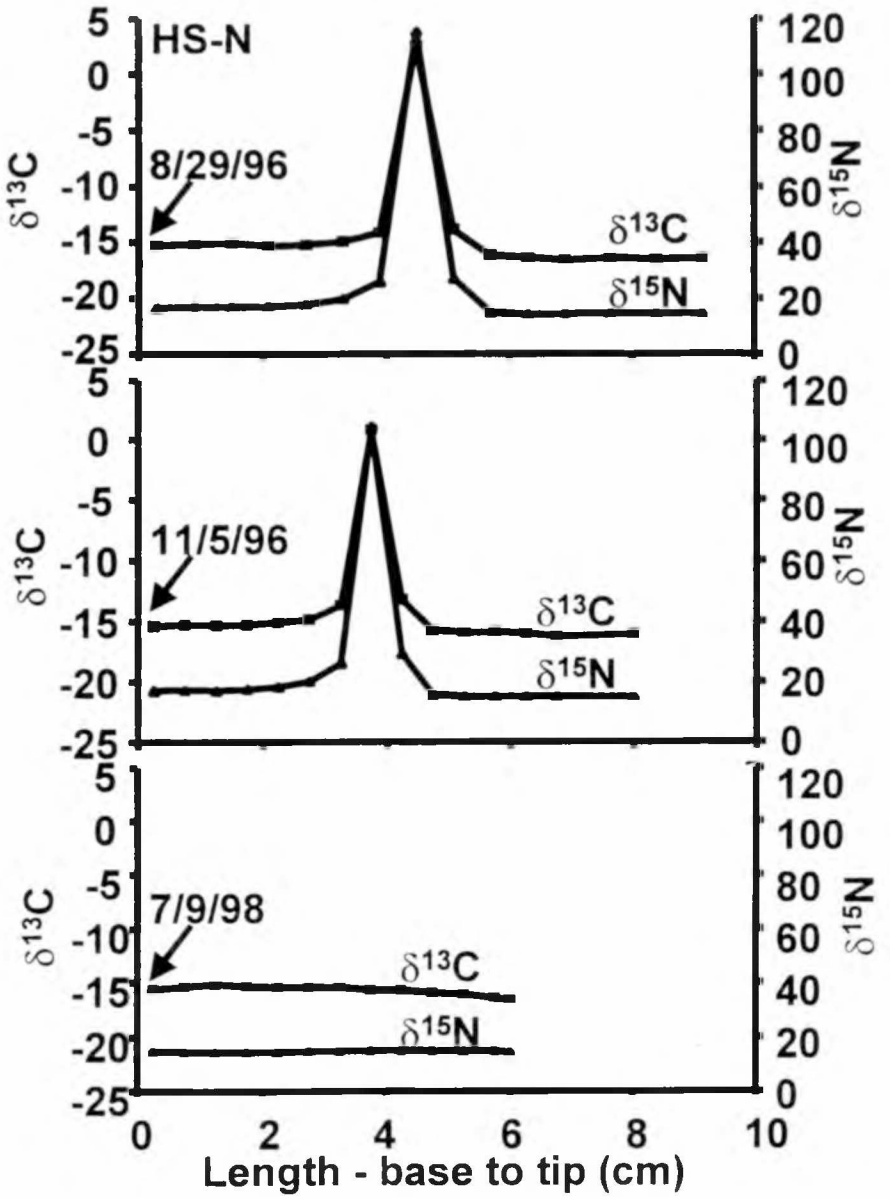

Figure 2.1. Stable isotope plots of vibrissae from a captive adult harbor seal, HS-N. The doubly labeled ($\delta^{13}C$ and $\delta^{15}N$) glycine peak is visible in the vibrissa cut in August 1996 (top). The vibrissa cut in November 1996 reveals the same peak in approximately the same location (middle). No peak is evident from a vibrissa cut in July 1998 (bottom). Plots show the most recent growth at the base of the vibrissae (0 cm).

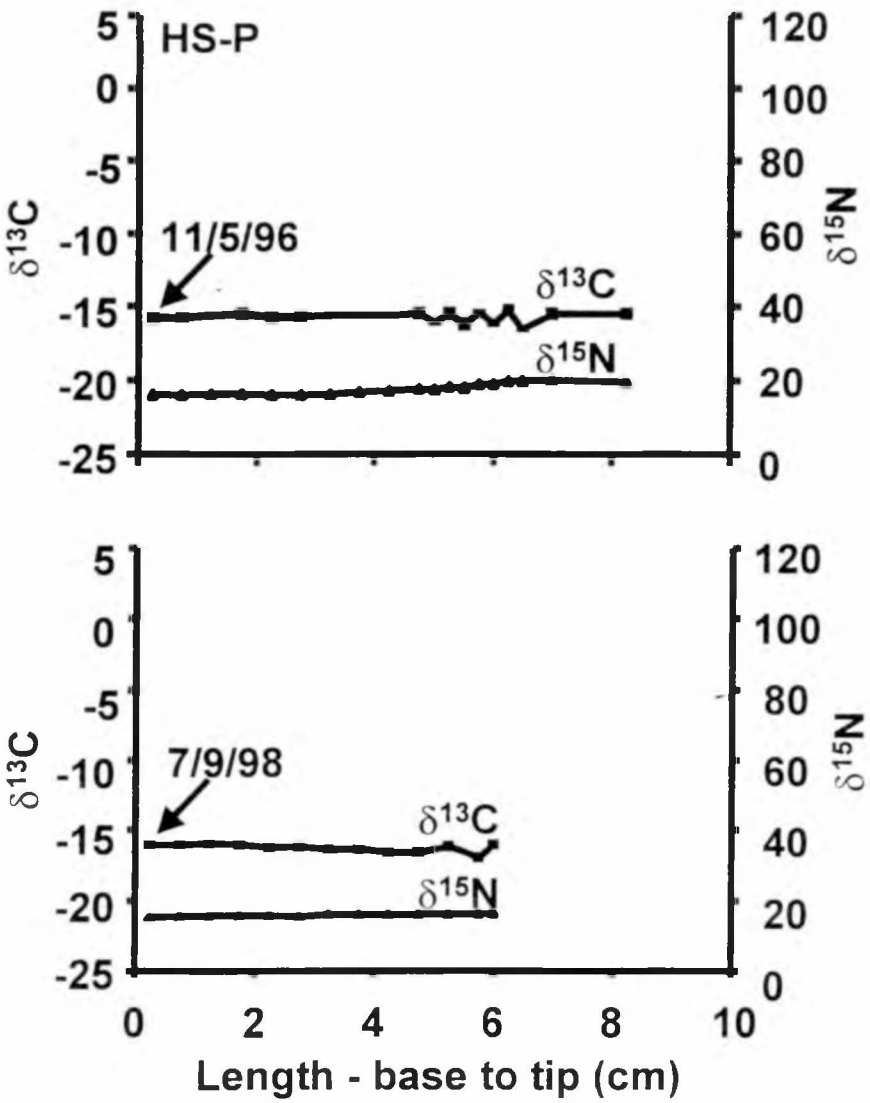

Figure 2.2. Stable isotope plots of vibrissae from a captive adult harbor seal, HS-P. No ^{15}N peak was visible in the vibrissa cut in November 1996 (top) or in the vibrissa cut in July 1998 (bottom). Plots show the most recent growth at the base of the vibrissae (0 cm).

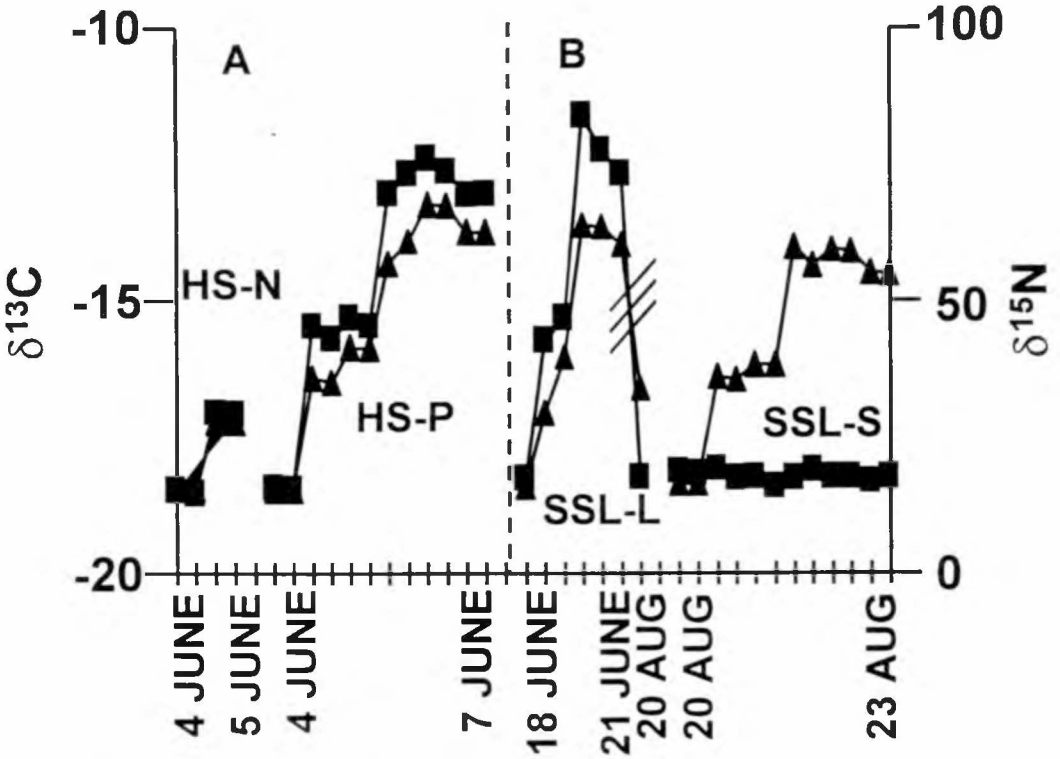

Figure 2.3. Stable isotope ratios in blood serum following injections of $\delta^{13}C$- and $\delta^{15}N$-glycine into (A) harbor seals HS-N and HS-P and (B) Steller sea lions SSL-L and SSL-S. Note the time scale break with SSL-L. SSL-S received only the ^{15}N label. (^{13}C – ■ and ^{15}N – ▲)

between the last administered label (June 1996) and the last cutting of the whiskers, 765 days later (July 1998). HS-N showed a significant increase in keratin $\delta^{15}N$ and $\delta^{13}C$, by 3‰ and 1.1‰, respectively, once the isotopic peak diminished and isotopic values returned to constant levels.

The decrease in blood serum isotope ratios in Figure 2.3 (SSL-L) showed evidence of the carbon isotope ratios returning to pre-injection levels sixty-four days later while the nitrogen isotope ratios in the serum showed a large enrichment equivalent to approximately 100% of the initial increase. This sixty-four day period was determined as the overall elapsed time between the initial increase and subsequent decrease in blood serum isotope ratios exhibited in SSL-L after the addition of the labeled glycine (refer to Figure 2.3). In the absence of harbor seal data, blood serum clearance information from the Steller sea lion was used. Due to the availability of the label, the time from the starting point of the increasing isotope ratios in HS-N's vibrissae until the point when the isotope ratios returned to constant levels was presumed to be sixty-four days. The enriched portion of the whisker measured approximately 2.1 cm and if the label were available for as long as 64 days, the period from mid-January to mid-March, the growth rate would be 0.33 mm/d. From the distance before and after the marker, HS-N's whiskers represented growth from late September 1995 to potentially mid-June 1996, assuming a constant growth rate. Growth had not resumed as of early November when the second whisker was analyzed as indicated by the same relative position of the marker in the vibrissae. If the isotopic

peak represented the label administered in January 1996, then the growth rate from the beginning of the peak until the time it was cut in August 1996 would be 0.37 mm/d. However, if the peak represented the label administered in June 1996, then the growth rate through August would have been 0.60 mm/d. The vibrissae growth rate based on the January injection date is nearly identical to the rate calculated from the rise and fall of the δ–values from the marker in the blood. These data would indicate that the peak resulted from the January injection of the label but that growth had ceased some time in June before the second label could be administered and incorporated. A third whisker removed from HS-N 20 months later showed no evidence of any carbon and nitrogen enrichment.

An adult harbor seal from southeastern Alaska that was originally tagged by the Alaska Department of Fish and Game and sampled in September 1994 was recaptured in April 1995. Whiskers that had been collected at both times were analyzed for their stable isotope ratios. During the seven months, the whiskers had an average growth rate of 0.08 mm/d (Figure 2.4). Neither the second nor the third recaptured harbor seals showed any similarity or overlap between their two vibrissae.

The subsistence-harvested adult seal showed no distinct difference in isotope ratios between anterior and posterior vibrissae and between vibrissae along the left and right sides of the muzzle (Figure 2.5). All the vibrissae ranged in length from 3 to 4 cm and eight vibrissae on each side of the muzzle were large enough to conduct isotopic analysis.

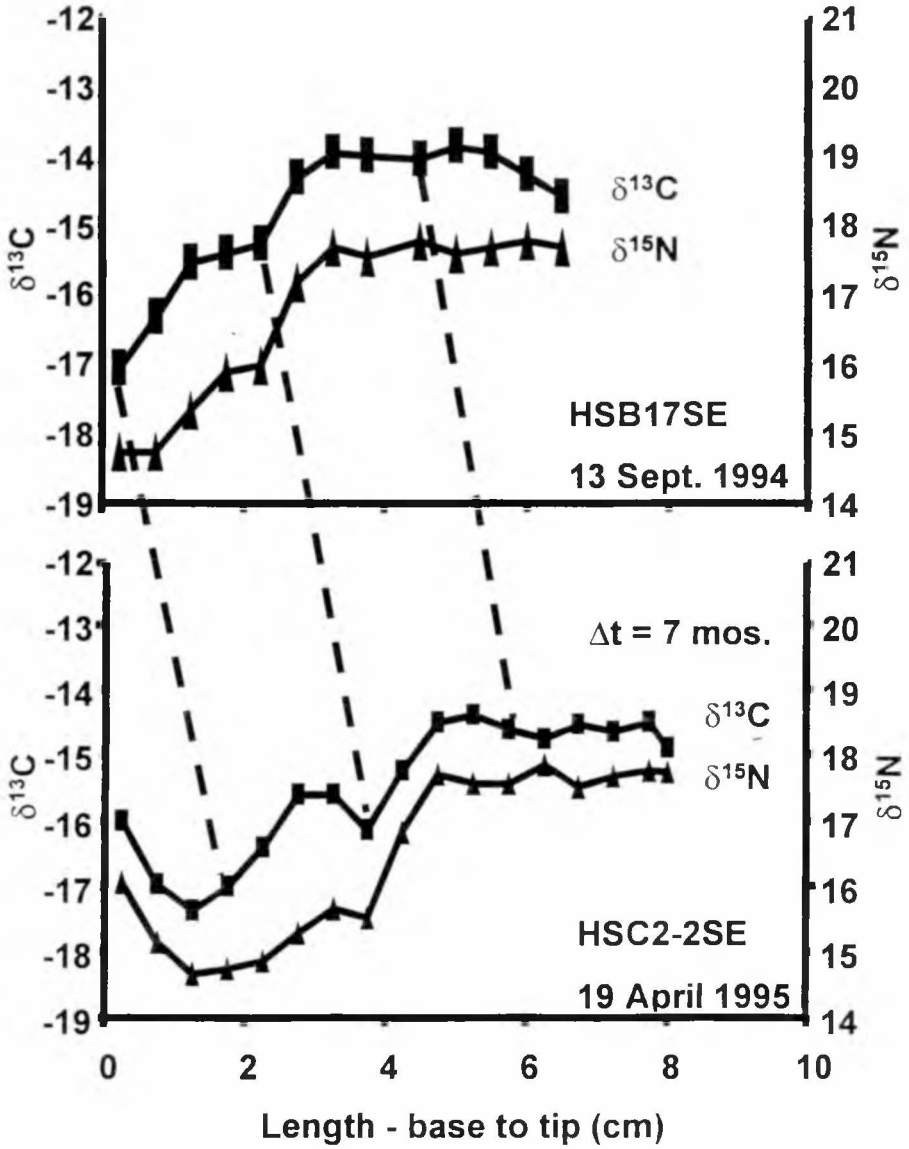

Figure 2.4. Stable isotope plots of vibrissae from a recaptured adult male harbor seal in southeastern Alaska. A vibrissa sampled in September 1994 (upper plot) is contrasted with a vibrissa taken from the same seals seven months later (lower plot). Plots show the most recent growth at the base of the vibrissae (0 cm).

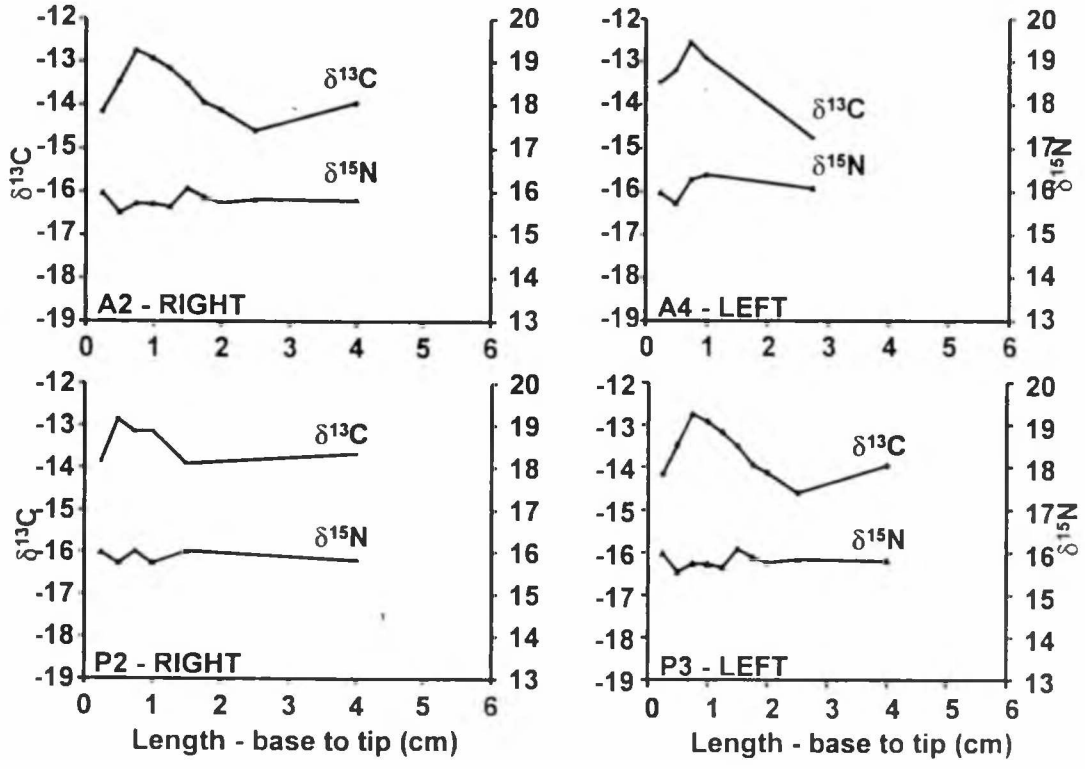

Figure 2.5. Stable isotope plots of vibrissae from a killed adult female harbor seal in Prince William Sound, Alaska. Anterior (top plots) and posterior (lower plots) vibrissae and left (left plots) and right-sided (right plots) vibrissae were contrasted. Plots show the most recent growth at the base of the vibrissae (0 cm).

Steller sea lions

The two captive adult sea lions each received two doses of labeled glycine as shown in Table 2.1. Whiskers were allowed to grow over a 672- and 735-day period, respectively. The first sea lion, SSL-L, received one dose of doubly labeled and one dose of ^{15}N-labeled amino acid 308 days apart. The stable isotope ratios in the vibrissae first showed the presence of both enriched $\delta^{15}N$ and $\delta^{13}C$ as peaks, corresponding to the doubly labeled amino acid, and later only a peak of enriched $\delta^{15}N$ corresponding to the ^{15}N-labeled amino (Figure 2.6). SSL-S's vibrissae exhibited two ^{15}N-enriched peaks that represented the two doses of ^{15}N-labeled glycine administered 183 days apart (Figure 2.7). Growth rates between the two markers ranged from 0.05 - 0.07 mm/d for both sea lions. The vibrissae were retained by the sea lions for more than 735 days demonstrating retention rates in excess of two years.

SSL-L showed a significant increase in keratin $\delta^{15}N$ and $\delta^{13}C$, by 2.4‰ and 0.6‰, respectively, after the enriched values began to diminish along the vibrissae and isotopic values returned to constant levels. No additional change in $\delta^{15}N$ was evident after the second $\delta^{15}N$ peak returned to a constant level. SSL-S showed an increase in keratin $\delta^{15}N$ by 0.8‰ after the first $\delta^{15}N$ peak returned to a constant level and also exhibited an additional 0.9‰ increase in $\delta^{15}N$ after the second isotopic peak diminished.

Two subadult Steller sea lions held at the Vancouver Aquarium in British Columbia, Canada, had their vibrissae clipped annually during a three-year period. It

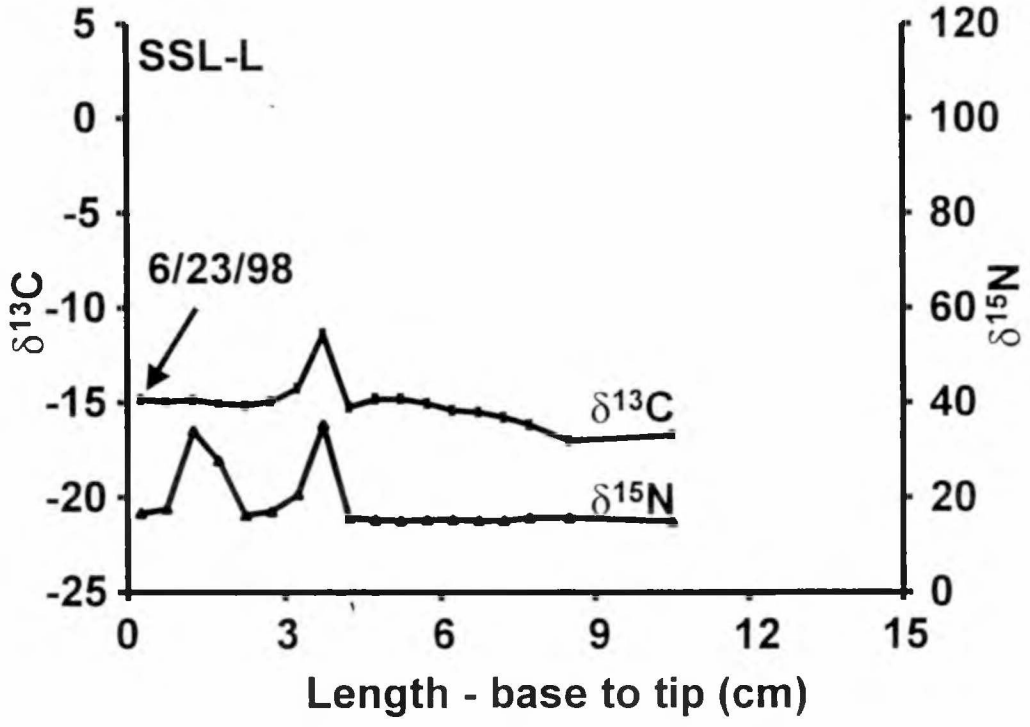

Figure 2.6. Stable isotope plot of a vibrissae from a captive adult Steller sea lion, SSL-L. The peaks furthest to the right in the $\delta^{13}C$ and $\delta^{15}N$ represent the doubly labeled glycine administered in June 1996 while the left peak in the $\delta^{15}N$ represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissa was cut in June 1998. Plots show the most recent growth at the base of the vibrissae (0 cm).

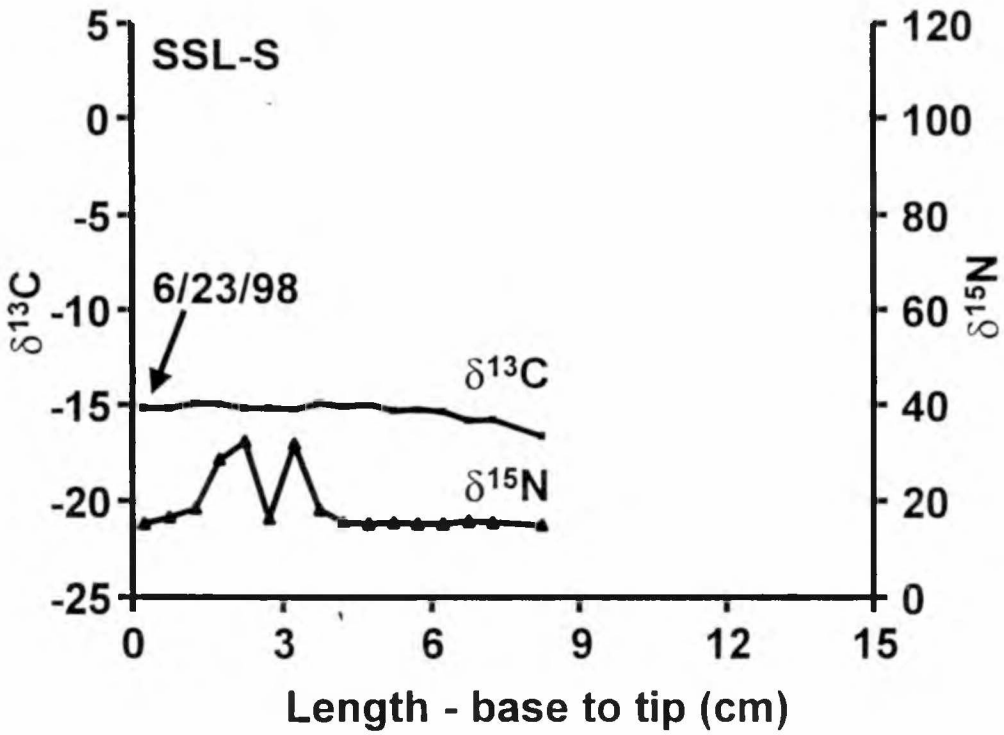

Figure 2.7. Stable isotope plot of a vibrissae from a captive adult Steller sea lion, SSL-S. The peak furthest to the right in the $\delta^{15}N$ represents the $\delta^{15}N$-labeled glycine administered in August 1996 while the left peak represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissa was cut in June 1998. Plots show the most recent growth at the base of the vibrissae (0 cm).

was possible to match features in the isotopic trends among one sea lion's vibrissae and the overlap in vibrissae allowed for a growth rate estimate. The daily growth rate for this animal, averaged over fourteen months, was 0.14 mm/d (Figure 2.8). A second sea lion had a much shorter overlap of growth in two successively cut whiskers. The daily growth rate for the second animal, averaged over two winter months, was 0.17 mm/d.

Subadult and adult sea lions (n = 30) sampled from the wild for another study had consistent isotopic oscillations along their vibrissae with growth rates ranging from 0.05 - 0.18 mm/d and averaging 0.10 - 0.14 mm/d, assuming that the major oscillations evident were annual (Figure 2.9). Oscillation length varied from animal to animal and year to year. Growth rates averaged over twelve months were 0.11 - 0.12 mm/d for all sea lions combined. The subsistence-harvested sea lion also showed no distinct variation in isotope ratios between anterior and posterior vibrissae and between vibrissae along the left and right sides of the muzzle (Figure 2.10).

DISCUSSION

These simple marker and observational studies indicate that the vibrissae growth characteristics between harbor seals and Steller sea lions are remarkably different. The growth rates in the seals indicated an irregular growth pattern throughout the year and annual vibrissae loss while the sea lions appeared to have a more consistent growth and year-to-year retention of their vibrissae (Table 2.2).

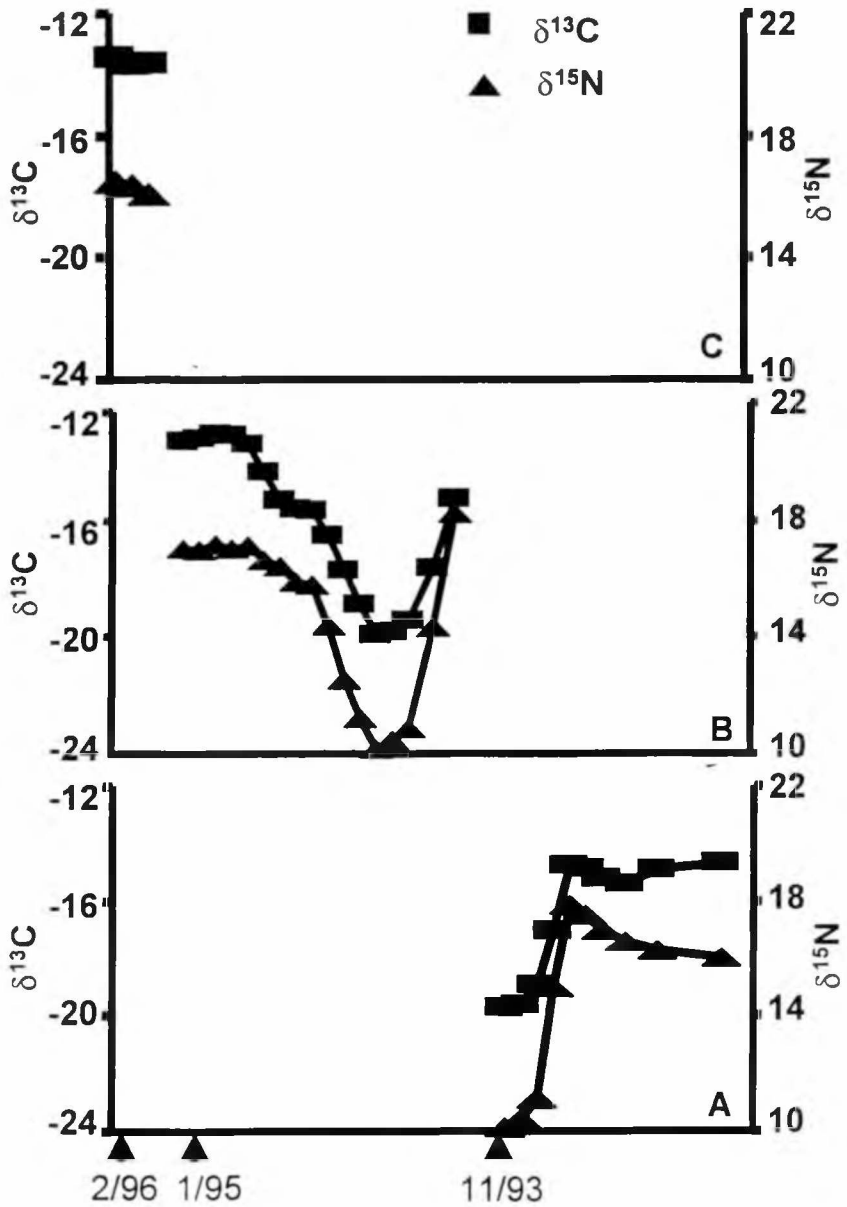

Figure 2.8. Stable isotope plots of vibrissae from a subadult female Steller sea lion in captivity. The first vibrissa (A) was cut in November 1993. The second vibrissa (B) was cut in January 1995 and shows overlap in growth with the first vibrissae. The third vibrissa (C) was cut in February 1996 and was too short to reveal any overlap in growth. Plots show the most recent growth at the base of the vibrissae (0 cm).

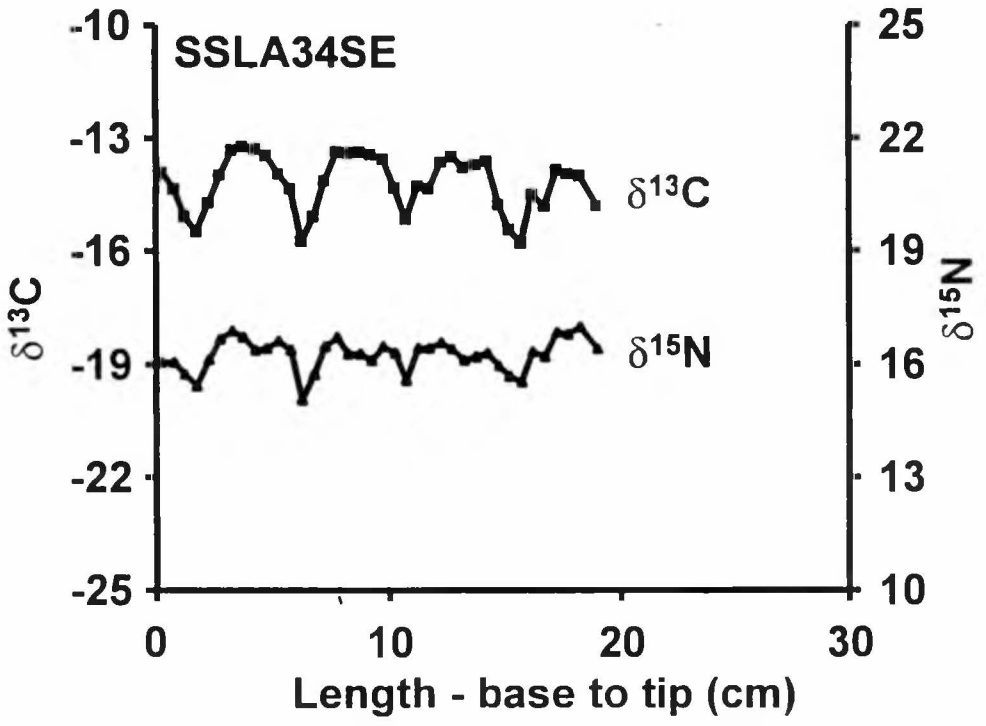

Figure 2.9. Stable isotope plot of a vibrissa from an adult female Steller sea lion in southeastern Alaska collected June 1993. Each oscillation represents one years' growth in the vibrissae. Plots show the most recent growth at the base of the vibrissae (0 cm).

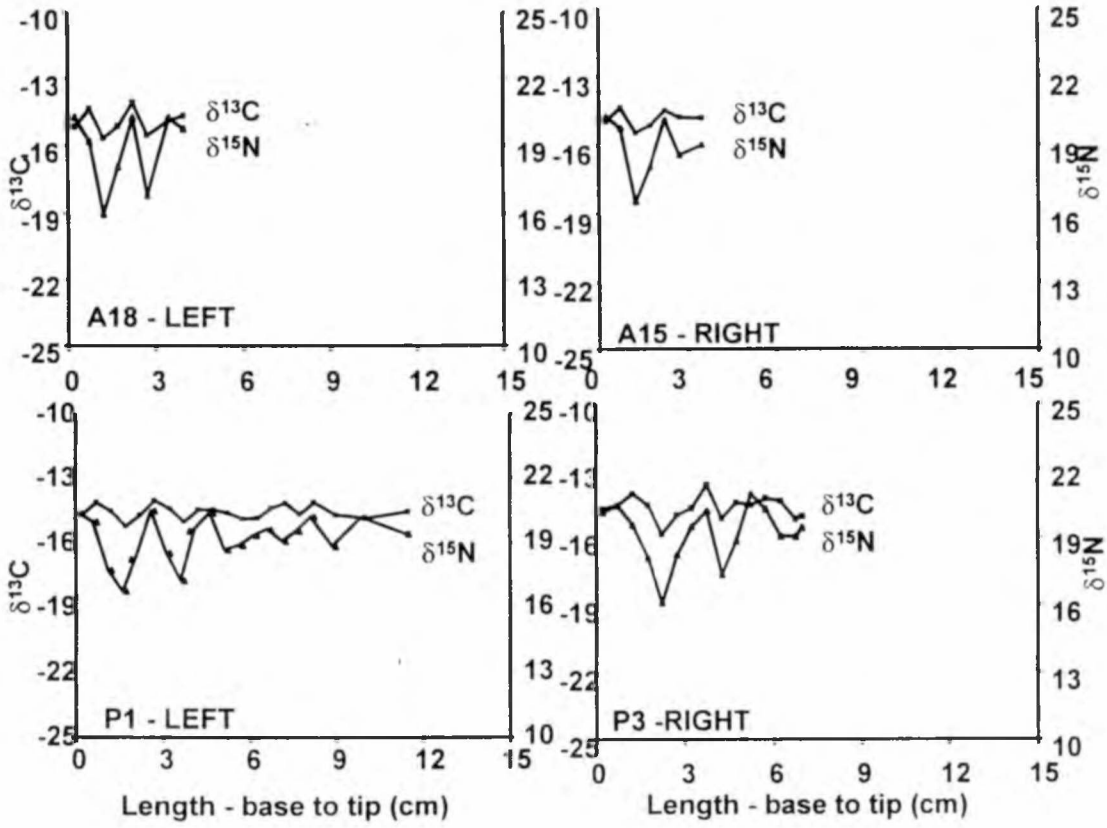

Figure 2.10. Stable isotope plots of vibrissae from a killed adult female Steller sea lion from the Pribilof Islands, Bering Sea, Alaska. Anterior (top plots) and posterior (lower plots) vibrissae and left (left plots) and right-sided (right plots) vibrissae were contrasted. Plots show the most recent growth at the base of the vibrissae (0 cm).

Table 2.2. Vibrissae growth rates in harbor seals and Steller sea lions.

Species	Location	Age	Mean growth (mm/d)
Harbor seals	captive (Mystic, CT)	adult	0.33 - 0.37
	wild (Alaska)	adult	0.08
Steller sea lions	captive (Mystic, CT)	adult	0.05 - 0.09
	captive (Vancouver)	subadult	0.14 - 0.17
	wild (Alaska)	adult	0.10 - 0.14

Vibrissae collected from seal HS-N appeared to have grown from the end of September until mid-June. HS-N had one isotopic peak after being given one dose of the labeled glycine in January but growth rate calculations indicate that the second dose given in June was not incorporated. Because the peak remained in approximately the same location on whiskers sampled from HS-N in August and November, this lent further support that the isotopic peak was the result of the January injection and that growth was assumed to have decreased to some minimal level or ceased altogether in June. At some time during the next twenty months, however, most or all of the whiskers appeared to have been lost and the marker was no longer evident in the vibrissae.

Variations in seasonal metabolic rates may have some connection to the period of rapid growth in the harbor seal vibrissae. Rosen and Renouf (1995) observed an 84% increase in the resting metabolic rates (RMR) of captive adult harbor seals from November through April and a higher than average RMR than the August estimates for the animals. The rapid growth in HS-N's vibrissae was also observed during the same winter-to-spring period.

The first measure we had of vibrissae growth rate in wild seals came from a recaptured adult prior to the beginning of the labeled experiment. During that seven-month period from September to April, vibrissae growth was thought to be constant at a rate one-fourth of the growth rate of the captive seal's vibrissae. One or both of these seals may have been impacted by differences in habitat, feeding and energetics

that could have affected their vibrissae growth rates. Further experimentation will be necessary to address these possibilities.

Both captive Steller sea lions that received two doses of singly and/or doubly labeled glycine showed evidence of the label in their vibrissae. The enriched isotope signals remained in the vibrissae more than two years after the initial doses of glycine were administered, which indicated that the vibrissae are retained from year-to-year. Vibrissae growth rates in captive adult sea lions had a range similar to the estimate rate for each oscillation in wild adult animals. Vibrissae from the Steller sea lions at the Vancouver Aquarium had been collected when the animals ranged in age from two to four years old. Periodic changes in the animals' diets are evident in the shifts in the stable isotopes along their vibrissae as represented by one sea lion in Figure 2.8. These changes were confirmed by isotopic analysis of the animals' food. The growth rate in the juvenile sea lions was twice the rate exhibited by the captive adults. Metabolic observations made by D. Rosen on the captive juvenile sea lions showed a maximum RMR during late fall and a minimum during April and May (per. comm.) but no comparable adult levels are available at this time. The oscillations observed in both the carbon and nitrogen isotope ratios of the wild sea lion in Figure 2.9 are not the result of endogenous rhythms but, rather, from dietary and geographic changes (this study). This point can be further illustrated by the lack of isotopic oscillations, except those resulting from the labeled glycine, in the sea lion vibrissae in Figures 2.6 and 2.7, which reflect the absence of any dietary changes during the experiment.

Stable isotope analysis of blood serum revealed that the carbon was cleared faster than the nitrogen. The carbon isotopes showed evidence of respiratory loss whereas the $\delta^{15}N$ values were additive over the duration of the experiment as a result of transamination and reincorporation into the body tissues. The pronounced changes in the nitrogen isotope ratios reflected the relative quantities of the element in the body composition of these animals. The nitrogen and carbon isotopes in the vibrissae keratin continued to show enrichment over the pre-injection values for a long time following the sharp, initial decline of the isotope peaks. The enrichment in HS-N's isotopes was most pronounced and led to a long-term increase of about 2‰ over the duration of the experiment. There were no changes in the animals' diets during or after the labeling experiment for the captive harbor seals and Steller sea lions. The total detectable retention time of the label remains unknown, as long-term monitoring of the animals' vibrissae could not be maintained. The residence time of this residual label, particularly in Steller sea lions that retain their vibrissae, should provide valuable information on turnover rates of proteins and could conceivably be used in wild animals where recapture is a possibility.

The carbon and nitrogen isotopes in the vibrissae of wild harbor seals did not show any type of repetitious pattern that might be indicative of an annual cycle. Two wild harbor seals recaptured one and two years apart, respectively, also displayed no similarity or overlap in the vibrissae stable isotope patterns between years. These data are consistent with the natural histories of the seals. The isotopic oscillations in the

vibrissae from wild adult Steller sea lions throughout the Bering Sea and Gulf of Alaska likely resulted from the movement and feeding of the animals in various geographic regions (Merrick et al. 1997; this study). The similar vibrissae growth rates in both wild and captive Steller sea lions, combined with the repetitive isotopic patterns in wild sea lions, provide evidence that sea lions retain their vibrissae for several years and likely replace them only when broken or worn.

Observations of northern elephant seal vibrissae made by Ling (1966) revealed that the vibrissae were not shed at the same time as the annual molt but replaced irregularly. Since vibrissae appear to function as individual sensory organs, any replacement because of loss or damage could be of greater selective advantage than regular seasonal changes (Ling 1977). Bowen (per. comm.) observed grey seals in captivity sporadically losing their vibrissae and rapidly re-growing them during the molting period. He has also observed the rapid re-growth of broken vibrissae on grey seals throughout the year. The captive Steller sea lions used in this growth experiment were observed rapidly re-growing the cut vibrissae while the remaining vibrissae showed no apparent change. Morphological differences exist between harbor seal and Steller sea lion vibrissae but it is unknown if those differences have any impact on the growth rate patterns in the vibrissae or vice versa. The wavy, or "beaded" surface of the harbor seal vibrissae differs from the smooth veneer of the Steller sea lion vibrissae. The lengths of a harbor seal's vibrissae (~ 10 cm) tend to be similar, while a

Steller sea lion's anterior mystacial vibrissae are short (~ 6 cm) compared to the much longer posterior vibrissae (>20 cm) (Hirons unpubl. data).

Both the empirical data and literature seem to support the idea that vibrissae growth rates and retention times vary among pinniped species but the cues, whether environmental and/or internal, remain unknown. Further experimentation on captive pinnipeds, supplemented by information from wild seals and sea lions, will be needed to better define these patterns. Stable isotope-labeled amino acids provide a safe and effective means of applying internal markers for vibrissae growth rate experiments.

Further studies should expand our understanding of how vibrissae growth may change throughout the animals' life span. Vibrissae from seals and sea lions contain a timeline of stable isotope ratios derived from prey items. By comparing the isotope ratios found along the lengths of vibrissae with the isotope ratios of suspected prey items, changes in food sources and habitat can be surmised for the temporal span represented by the growth of the whisker. The trophic information these tissues provide will enhance our knowledge of the animals' food resources while perhaps providing clues to their population declines.

ACKNOWLEDGMENTS

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the trainers at the Vancouver Aquarium assisted in the collection of whiskers from sea lions. Norma Haubenstock and Bruce Barnett assisted in the stable isotope analyses. This research was authorized under US Marine Mammal Protection Act Permit 802 issued by the National Marine Fisheries Service, and conducted with funds from the *Exxon Valdez* Oil Spill Trustees Council, the Coastal Marine Institute of Minerals Management Service under contract # 14-35-0001-30661, the North Pacific Universities Consortium on Marine Mammal Research and the Rasmuson Fisheries Research Center. This is contribution no. xxx from the Sea Research Foundation.

CHAPTER 3

STABLE ISOTOPE RATIOS IN PINNIPED VIBRISSAE AS FOOD WEB TRACERS

ABSTRACT

Stable isotope ratios in vibrissae (whiskers) of Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) revealed a regular oscillating pattern in both carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) that repeated along the length of the vibrissae. These isotope ratios were compared with prey species in several regional food webs in the Bering Sea, Gulf of Alaska and northeastern Pacific Ocean. By combining recorded observations of these species' movements with food web data, a picture of the foraging patterns by the animals can be estimated from isotope ratios recorded in their vibrissae. Stable isotope ratios from northern fur seal vibrissae were consistent with a diet of predominantly squid and juvenile walleye pollock. The vibrissae from Bering Sea and western Gulf of Alaska Steller sea lions had isotope ratios consistent with a diet of gadids (both Pacific cod and walleye pollock) and some Atka mackeral from the Bering Sea, Alaska Peninsula and Kodiak. The isotope ratios in vibrissae from some sea lions had no corresponding prey from the list of sampled organisms.

Hirons AC, Schell DM, Springer AM (in prep) Stable isotope ratios in pinniped vibrissae as food web tracers. Can J Fish Aquat Sci

INTRODUCTION

Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) inhabit waters of the southern Bering Sea and the Gulf of Alaska, where their populations have declined during the past three decades (Loughlin et al. 1992; Trites and Larkin 1996; Sinclair 1997; Strick et al. 1997), beginning in the eastern Aleutian Islands and spreading into the western Aleutians, the eastern Bering Sea and the Gulf of Alaska (Alaska Sea Grant 1993). Steller sea lions travel hundreds of kilometers each year as they move between rookeries for breeding and pupping, haul-out sites and feeding locations. The majority of the northern fur seal population migrates annually from rookeries in the Bering Sea to forage locations ranging from the Gulf of Alaska, into open waters of the Pacific Ocean and as far south as California. The greatest abundance of sea lions is found in the Gulf of Alaska, although high numbers have been spotted in the central Bering Sea during the winter (Loughlin 1993). Feeding appears to occur predominantly onshelf and along the edge of the continental shelf.

The feeding grounds within 200-300 km of the Pribilof Islands are important for the fur seals during the duration of their breeding and molting (Loughlin et al. 1987) and the passes around the Aleutian Islands are important foraging grounds when the seals are migrating to and from the Pribilof Islands (Bigg 1990). The greatest numbers of fur seals have been reported along the continental shelf and slope in the Bering Sea and Gulf of Alaska, presumably due to abundant food (Kajimura 1985).

Decreased availability of preferred prey species and size classes is believed to be a factor in the pinniped declines (Alverson 1991, Springer 1992, Trites 1992, Merrick 1995, Merrick et al. 1997). Wild pinnipeds generally have a varied diet but will emphasize a few abundant prey species. Abundances tend to vary with different geographic locations (Fiscus and Baines 1966; Pitcher 1981; Perez and Bigg 1986). Walleye pollock, Atka mackeral, Pacific cod, capelin, herring, sandlance and squid have been identified as important in seal and sea lion diets through the identification of prey remains (otoliths, bony parts) in the stomachs of these animals. The absence of soft-bodied prey or presence of bony parts from prey may or may not represent prey assimilated by the seal or sea lion. (Pitcher 1980, 1981; Lowry 1982, Kajimura 1985; Lowry et al. 1989; Merrick 1995; Merrick et al. 1997). Dietary information has often been dependent upon feeding observations or scat and stomach content analyses that may have been biased by very short observation times.

The stable isotope ratios of carbon ($^{13}C/^{12}C$) and, perhaps more importantly, nitrogen ($^{15}N/^{14}N$) show a stepwise enrichment with each increasing trophic level in the marine environment (DeNiro and Epstein 1978, 1981). Carbon and nitrogen isotope ratios increase by 1‰ and 3‰, respectively, with each trophic step in food webs of the North Pacific and Arctic oceans (McConnaughey and McRoy 1979; Fry 1988; Hobson and Welch 1992; Hobson et al. 1997).

Muscle tissue tends to reflect the average isotopic composition of the entire animal (DeNiro and Epstein 1978, 1981) while other body tissues are generally isotopically enriched or depleted in comparison (Tieszen et al.1983). Assuming the

isotope ratios for muscle tissue were typical of whole animal values, ratios from vibrissae are normalized to muscle from the same species and compared directly with the isotope ratios of prey species muscle. The turnover rates of carbon and nitrogen vary according to the metabolic rate of each tissue so it is possible to have different tissues reflect isotope ratios for different temporal periods (weeks vs. years) (Hobson and Clark 1992; Hobson et al. 1996; Ben-David 1996). Schell et al. (1989) have shown that for whale baleen, a continuously growing tissue, the stable isotope ratios along the lengths of the baleen plates provided dietary information for many years. This technique has also been applied in the present study, using vibrissae to reveal information on pinniped diets over time.

Stable carbon isotope ratios can be used to distinguish different marine food webs by identifying different sources of organic matter at the base of each web (reviewed by Fry and Sherr 1984; Minagawa and Wada 1984; Saupe et al. 1989; Simenstad et al. 1993). Herbivorous zooplankton are the first-order consumers of primary productivity and integrators of seasonal carbon isotope values of the phytoplankton. These values are then passed through the food web to higher order consumers.

Isotope ratio gradients of zooplankton along the coast of the Beaufort Sea have been described by Dunton et al. (1985, 1989) and Saupe et al. (1989). Schell et al. (1998) compiled those data and identified regions of distinct isotopic signatures. Schell (pers.comm.) also defined isotope gradients along the coastline in the Gulf of Alaska that are enriched inshore and become more depleted offshore. Similar

differences in $\delta^{13}C$ were found off the coast of British Columbia between the shelf and the adjacent slope (Perry et al. 1999). These regions appear to be defined by physical characteristics that either enhance or reduce the primary productivity (Springer et al. 1996; Schell et al. 1998). This isotopic information is necessary to interpret the spatial sequencing of stable isotope values for pinniped species that have wide ranges or migration patterns that include foraging in several food webs. Regional differences affecting prey isotope ratios were used to help locate areas of foraging for seals and sea lions traveling great distances.

MATERIAL AND METHODS

Sampling protocol

From 1993 to 1998 pinniped and prey samples were collected from the southeastern Bering Sea, the Gulf of Alaska and the northeastern Pacific Ocean with assistance from Native hunters and state and federal agencies (Figure 3.1). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in vibrissae (whiskers) and muscle were measured in 14 northern fur seals and 22 Steller sea lions from the Pribilof Islands in the Bering Sea and 14 Steller sea lions from Chirikof Island in the western Gulf of Alaska. One to two vibrissae were cut or pulled from live animals while dead animals had all available vibrissae removed. One cubic centimeter of unexposed muscle tissue was collected from dead animals and frozen.

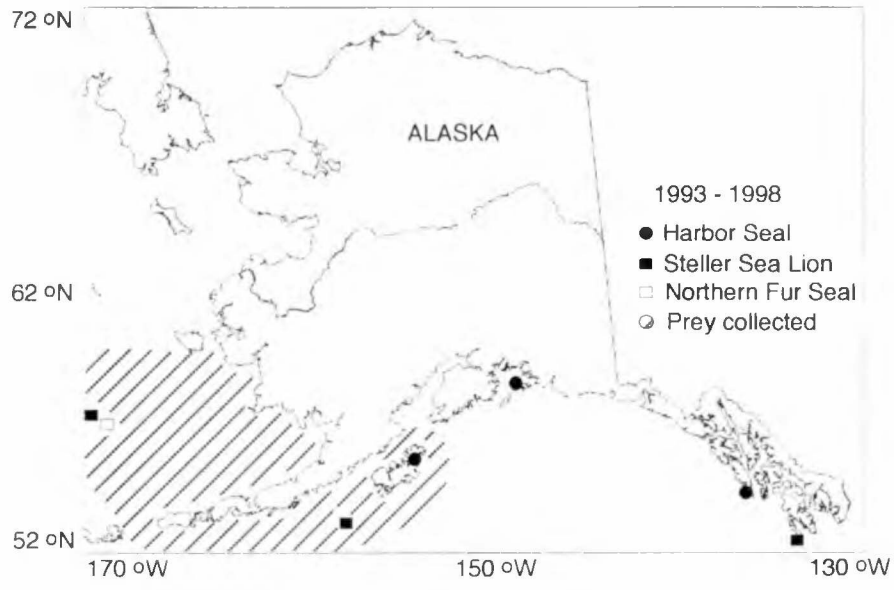

Figure 3.1. Locations of sample sites for pinnipeds and prey species in the southeastern Bering Sea and Gulf of Alaska, 1993-1998.

Samples of suspected prey species were obtained during cruises in the Gulf of Alaska and southern Bering Sea in the summers of 1993-1998. The samples from the Bering Sea were collected during the National Marine Fisheries Service (NMFS) annual ground fish surveys; those from the Gulf of Alaska were collected during Alaska Department of Fish and Game (ADFG) and NMFS surveys. Three hundred individual fish and cephalopods were sampled for stable isotope ratios. Muscle tissue was taken from the lateral sides of fish and from the mantles of cephalopods. Isotopic values of zooplankton from the Bering Sea and Gulf of Alaska and of prey items from the coastal waters of Washington and Oregon were obtained from literature sources (Hobson et al. 1997; Schell et al. 1998; this study).

Mass spectrometry

Muscle tissue was kept frozen between collection of the tissue and preparation for isotope analysis. Muscle samples were dried in a laboratory oven at 60°C for a minimum of forty-eight hours and ground for homogeneity. Vibrissae were scrubbed with steel wool to remove any debris and segmented from base to tip in 2.5 mm segments. Every other segment beyond the base was analyzed for carbon and nitrogen isotope ratios and the reserved pieces were kept for additional detail as needed.

Subsamples of muscle and vibrissae (1-2 mg) were combusted at high temperature using a Europa Roboprep CHN analyzer and the nitrogen and carbon gases were separated and purified by gas chromatography. All samples were then analyzed for stable isotope ratios in a Europa 20/20 continuous flow isotope ratio

mass spectrometer (CF-IRMS). All samples, except the vibrissae, had duplicates analyzed. Stable isotope ratios were expressed in the following standard notation:

$$\delta X\ (‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively. $R_{standard}$ for ^{13}C is Pee Dee Belemnite and for ^{15}N is atmospheric N_2 (air). Analytical error for samples was approximately ± 0.2‰ for both carbon and nitrogen.

Statistical analysis

Analysis of variance (ANOVA) was used to distinguish isotopic differences among prey species in spatially distinct food webs. Food web regions were designated based upon the availability of samples from several prey species within one geographic area. Significant differences among regions were determined at the P = 0.05 level. The isotopic differences between muscle and vibrissae from each pinniped species were analyzed using least squares means and those values were used to normalize the vibrissae isotope ratios to muscle (SYSTAT 1997). Due to the limited samples of female or male fur seals and sea lions from an area and limited age distribution, sex and age differences could not be tested.

RESULTS

Isotope ratios of prey species

Herring, capelin, pollock and Pacific cod collected along the Bering Sea shelf break were not isotopically different from the same species on the inner shelf, with differences ranging less than 0.8‰ in the average $\delta^{13}C$ and not more than 1.8‰ in the average $\delta^{15}N$. In contrast, the copepod $\delta^{13}C$ values were more than 2‰ depleted on the inner shelf versus the shelf break. Since pelagic species range throughout many of the isotopically defined regions, food may be derived from each region. Pacific cod, walleye pollock and herring found along the Bering Sea shelf break had lower average $\delta^{13}C$ values (1-2‰), while average $\delta^{15}N$ values were almost the same as those for the same species found along the Aleutian Shelf. The one difference in the average $\delta^{15}N$ was that adult herring from the Bering Sea were almost 2‰ lower than the same species found along the Aleutian Shelf. Excluding shrimp and squid, less than 1‰ difference in $\delta^{13}C$ was found between prey from the Aleutian Shelf and prey from Kodiak waters. The $\delta^{15}N$ values also ranged less than 1‰ for all species except arrowtooth flounder, shrimp and squid.

In comparing the ratios of both isotopes for prey from the inner shelf and shelf break of the Bering Sea, we found no significant differences in isotope ratios between the two food webs except in the $\delta^{13}C$ of the calanoid copepods ($P \leq 0.001$). The prey species from the shelf break were then compared with the same species from along the Aleutian Shelf and significant differences were found between adult herring for

$\delta^{13}C$ and $\delta^{15}N$ ($P = 0.008$, $P = 0.002$). Arrowtooth flounder from waters surrounding the Aleutian Islands were significantly different in $\delta^{15}N$ ($P \leq 0.001$) from arrowtooth flounder in the waters around Kodiak Island. The mean $\delta^{13}C$ and $\delta^{15}N$ values were also different in squid ($P = 0.038$, $P = 0.017$) and in shrimp ($P = 0.018$, $P \leq 0.001$) between these two areas. Sole sampled along the Bering Sea shelf break had higher $\delta^{13}C$ values than anticipated based on their $\delta^{15}N$, an indicator of trophic level. Sole and shrimp along the southern side of the Alaska Peninsula and around Kodiak Island had higher $\delta^{13}C$ values than expected based on their $\delta^{15}N$ values (Table 3.1).

Isotope ratios of pinniped species

The isotopic ratios of vibrissae and muscle from sixteen Steller sea lions and three northern fur seals were compared to determine fractionation differences between these tissues. Using least square means, northern fur seal vibrissae were enriched by 1.6‰ in the $\delta^{13}C$ relative to muscle while Steller sea lion vibrissae were enriched by 1.8‰. Steller sea lion and northern fur seal vibrissae were depleted by 0.6‰ in $\delta^{15}N$ relative to muscle (Table 3.2).

The most enriched $\delta^{13}C$ and $\delta^{15}N$ values along the vibrissae were labeled "MAX" and the most depleted values were labeled "MIN". There had to be a difference of $\geq$ 1‰ in $\delta^{13}C$ and $\geq$ 3‰ in $\delta^{15}N$ between the maximum and minimum values for these points to be identified (Figures 3.2-3.4).

The regular, oscillating isotope patterns found along the vibrissae of northern fur seals and Steller sea lions imply seasonal movement and feeding in isotopically

Table 3.1. Mean (± SE) $\delta^{13}C$ and $\delta^{15}N$ values measured in muscle of fishes and invertebrates from the southeastern Bering Sea and Gulf of Alaska.

Species	**Location**	**n**	$\delta^{13}C$	$\delta^{15}N$
copepods	Bering Sea - inner shelf	6	-23.2 ± 0.2	8.0 ± 0.2
copepods	Bering Sea - shelf break	5	-21.1 ± 0.3	8.1 ± 0.2
copepods	Alaska Peninsula	6	-20.9 ± 0.2	9.0 ± 0.3
copepods	Kodiak	6	-21.0 ± 0.3	9.1 ± 0.2
Pacific cod, adult	Bering Sea - inner shelf	5	-17.4 ± 0.2	16.7 ± 0.5
Pacific cod, adult	Bering Sea - shelf break	4	-16.9 ± 0.5	16.1 ± 0.7
Pacific cod, adult	Alaska Peninsula	28	-16.4 ± 0.1	16.0 ± 0.2
Pacific cod, adult	Kodiak	33	-16.8 ± 0.1	15.7 ± 0.2
walleye pollock, ad.	Bering Sea - inner shelf	7	-19.0 ± 0.2	14.8 ± 0.5
walleye pollock, ad.	Bering Sea - shelf break	5	-18.8 ± 0.1	13.0 ± 0.7
walleye pollock, ad.	Alaska Peninsula	18	-17.9 ± 0.2	14.2 ± 0.3
walleye pollock, ad.	Kodiak	36	-18.1 ± 0.1	13.2 ± 0.2
walleye pollock, juv.	Bering Sea - inner shelf	4	-19.2 ± 0.3	13.2 ± 0.5
walleye pollock, juv.	Bering Sea - shelf break	3	-18.8 ± 0.7	13.1 ± 0.9
walleye pollock, juv.	Alaska Peninsula	35	-18.3 ± 1.3	13.4 ± 1.4
walleye pollock, juv.	Kodiak	0	–	–
herring, adult	Bering Sea - inner shelf	2	-20.8 ± 0.9	13.0 ± 1.9
herring, adult	Bering Sea - shelf break	3	-20.7 ± 0.7	12.2 ± 0.6
herring, adult	Alaska Peninsula	6	-18.6 ± 0.2	14.3 ± 0.1
herring, adult	Kodiak	20	-19.2 ± 0.2	14.7 ± 0.1
herring, juvenile	Bering Sea - inner shelf	0	–	–
herring, juvenile	Bering Sea - shelf break	0	–	–
herring, juvenile	Alaska Peninsula	7	-20.4 ± 0.3	12.3 ± 0.6
herring, juvenile	Kodiak	0	–	–
capelin, adult	Bering Sea - inner shelf	17	-20.2 ± 0.2	13.8 ± 0.1
capelin, adult	Bering Sea - shelf break	8	-19.3 ± 0.3	14.1 ± 0.3
capelin, adult	Alaska Peninsula	0	–	–
capelin, adult	Kodiak	5	-20.7 ± 0.4	15.5 ± 0.2
capelin, juvenile	Bering Sea - inner shelf	0	–	–
capelin, juvenile	Bering Sea - shelf break	0	–	–
capelin, juvenile	Alaska Peninsula	6	-21.0 ± 0.1	12.6 ± 0.2
capelin, juvenile	Kodiak	6	-21.0 ± 0.1	12.6 ± 0.2

Table 3.1 – cont.

Species	Location	n	$\delta^{13}C$	$\delta^{15}N$
sole (spp.)	Bering Sea - inner shelf	0	–	–
sole (spp.)	Bering Sea - shelf break	12	-16.6 ± 0.2	14.7 ± 0.2
sole (spp.)	Alaska Peninsula	5	-16.5 ± 0.1	14.1 ± 0.3
sole (spp.)	Kodiak	6	-15.6 ± 0.1	14.4 ± 0.3
Atka mackeral, ad.	Bering Sea - shelf break	2	-22.3 ± 0.1	11.8 ± 0.1
Atka mackeral, ad.	Alaska Peninsula	2	-21.5 ± 0.1	12.5 ± 0.1
arrowtooth flounder	Alaska Peninsula	5	-19.0 ± 0.3	12.2 ± 0.1
arrowtooth flounder	Kodiak	5	-18.6 ± 0.1	16.3 ± 0.2
shrimp (spp.)	Alaska Peninsula	5	-17.6 ± 0.1	12.1 ± 0.1
shrimp (spp.)	Kodiak	4	-18.6 ± 0.4	17.4 ± 0.1
squid (spp.)	Alaska Peninsula	4	-20.1 ± 0.4	11.9 ± 0.2
squid (spp.)	Kodiak	3	-18.9 ± 0.1	13.8 ± 0.6
octopus (spp.)	Bering Sea - shelf break	8	-17.7 ± 0.1	13.1 ± 0.2

Table 3.2. $\delta^{13}C$ and $\delta^{15}N$ fractionation differences between pinniped vibrissae and muscle using least square means (± SE). BS is Bering Sea and GOA is Gulf of Alaska.

Species	**Location**	**n**	**$\delta^{13}C$**	**$\delta^{15}N$**
Northern fur seals	Pribilof Islands, BS	3	1.6 ± 0.3	-0.6 ± 0.3
Steller sea lions	Pribilof Islands, BS & Chirikof Island, GOA	16	1.8 ± 0.2	-0.6 ± 0.2

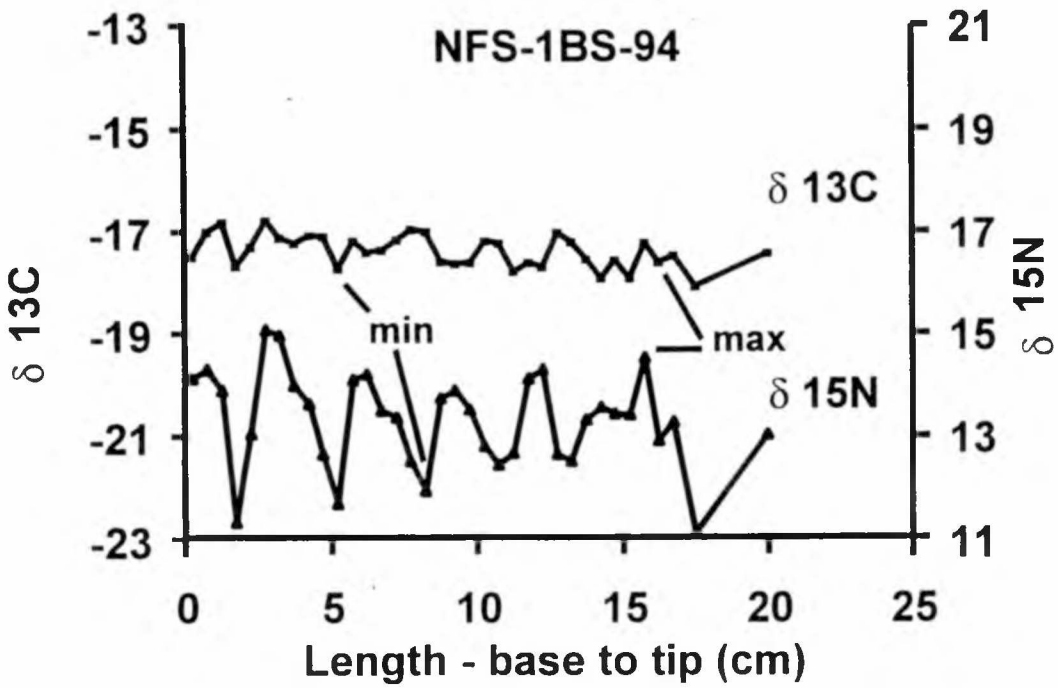

Figure 3.2. $\delta^{13}C$ and $\delta^{15}N$ in a northern fur seal vibrissa from the Pribilof Islands, Bering Sea, July 1994. The base of the vibrissa begins at 0 cm. Examples of maximum and minimum isotope values are labeled.

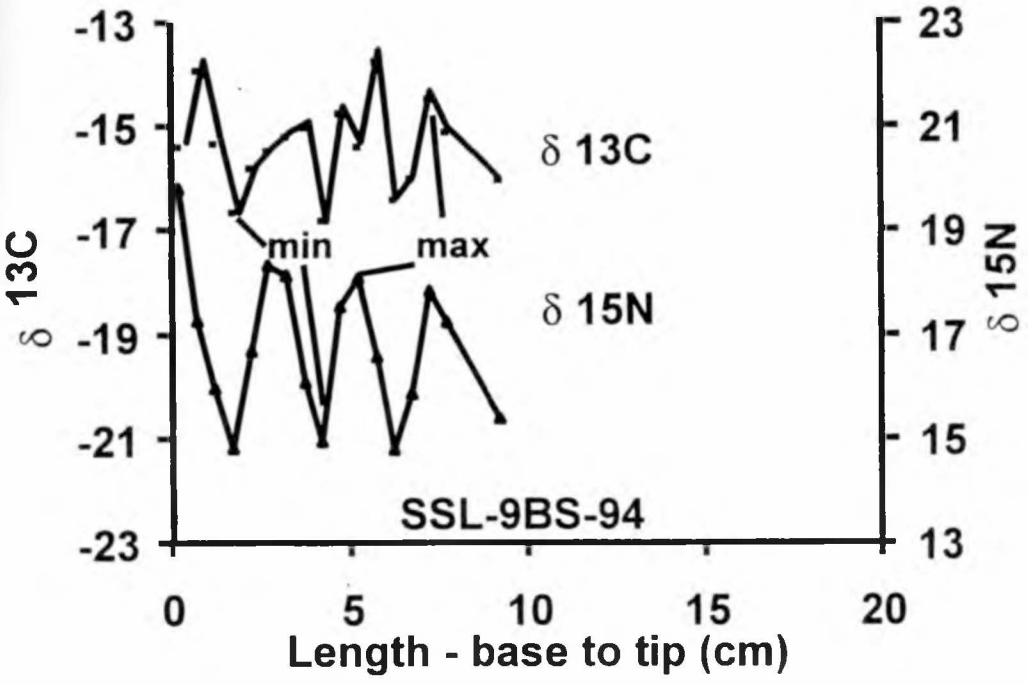

Figure 3.3. $\delta^{13}C$ and $\delta^{15}N$ in a Steller sea lion vibrissa from the Pribilof Islands, Bering Sea, May 1994. The base of the vibrissa begins at 0 cm. Examples of maximum and minimum isotope values are labeled.

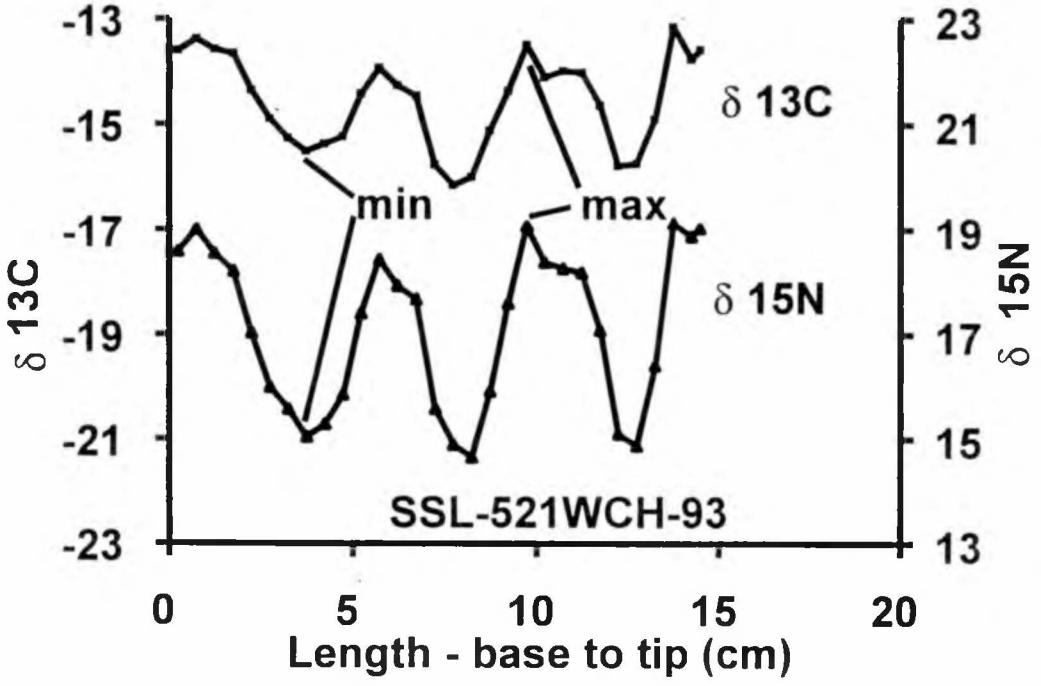

Figure 3.4. $\delta^{13}C$ and $\delta^{15}N$ in a Steller sea lion vibrissa from Chirikof Island, Gulf of Alaska, July 1993. The base of the vibrissa begins at 0 cm. Examples of maximum and minimum isotope values are labeled.

distinct regions (Schell et al. 1998; Perry et al 1999). Northern fur seals are seasonally migratory and sea lions have large ranges that cover several hundred kilometers (Kenyon and Rice 1961; Kajimura 1985; Loughlin et al. 1984). Northern fur seals were sampled in the Pribilof Islands of the Bering Sea and all had consistent oscillating patterns in both $\delta^{13}C$ and $\delta^{15}N$ (Figure 3.2). The periodicity of these patterns ranged from 1-3 cm along the vibrissae. Bering Sea fur seals had average maximum ratios ranging from 14.0 to 17.4‰ for $\delta^{15}N$ and -17.3 to -16.1‰ for $\delta^{13}C$ and average minimum ratios ranging from 12.0 to 14.5‰ for $\delta^{15}N$ and -18.2 to -17.3‰ for $\delta^{13}C$ (Table 3.3).

The sea lion isotope patterns were characterized either by periodic oscillations of amplitude ranging from less than 2‰ to over 5‰ or by patterns that were characterized by low amplitude oscillations having larger trends toward isotope enrichment or depletion (Figure 3.3 and 3.4). The stable isotope ratios of Chirikof Island Steller sea lions demonstrated the most regular oscillating patterns in $\delta^{13}C$ and $\delta^{15}N$ with periods ranging from 3 - 6 cm along the whisker. Bering Sea sea lions had less regular oscillations than those from the Gulf of Alaska. The Bering Sea sea lions had average maximum values ranging from 15.7 to 21.0‰ for $\delta^{15}N$ and -16.8 to -14.2‰ for $\delta^{13}C$ and average minimums ranging from 14.4 to 18.2‰ for $\delta^{15}N$ and -17.3 to -14.9‰ for $\delta^{13}C$ (Table 3.3).

The maximum isotope values of the male fur seal vibrissae were consistent with a diet of predominantly squid and juvenile walleye pollock from waters around

Table 3.3. $\delta^{13}C$ and $\delta^{15}N$ values for pinniped vibrissae from the Pribilof Islands, Bering Sea and from Chirikof Island, Gulf of Alaska. Age of the animals are denoted by A (adults), SA (subadults), P (pups) and U (unknown).

Pinniped	Sample Date	Sex	Age	Range $\delta^{13}C$	$\bar{x}\ \delta^{13}C$	Range $\delta^{15}N$	$\bar{x}\ \delta^{15}N$
Northern fur seals - Pribilof Islands, Bering Sea							
NFSA1	29 June 1993	M	A	-17.7 to -16.1	-16.9	13.5 to 16.0	14.9
NFSA2	12 Aug. 1993	F	A	-17.6 to -15.8	-16.9	13.1 to 16.7	15.1
NFSA3	15 Aug. 1993	M	A	-18.2 to -17.1	-17.6	12.6 to 15.1	13.8
NFSA4	June 1993	M	U	-18.1 to -16.9	-17.5	11.3 to 16.7	12.8
NFSA5	June 1993	M	U	-18.1 to -17.1	-17.5	11.5 to 17.6	13.3
NFSA6	June 1993	M	U	-18.7 to -16.8	-17.5	10.7 to 17.4	13.6
NFSA7	June 1993	M	U	-18.6 to -16.5	-17.4	10.9 to 17.6	13.6
NFSB1	26 July 1994	M	SA	-18.1 to -16.8	-17.4	11.1 to 15.1	13.3
NFSB2	5 Aug. 1994	M	SA	-18.1 to -15.9	-17.0	11.9 to 18.8	15.4
NFSB3	11 July 1994	M	SA	-19.4 to -16.6	-17.6	11.6 to 18.6	14.3
NFSB4	16 July 1994	M	SA	-18.1 to -16.1	-16.7	11.8 to 17.9	15.0
NFSB5	15 July 1994	M	SA	-19.5 to -16.2	-17.7	10.9 to 19.0	14.4
NFSB6	5 Aug. 1994	M	SA	-18.2 to -16.1	-17.2	12.8 to 18.1	15.5
NFSB7	5 Aug. 1994	M	SA	-16.5 to -17.8	-17.2	12.8 to 19.7	15.6
Steller sea lions - Pribilof Islands, Bering Sea							
SSLA1BS	30 May 1993	M	SA	-18.5 to -13.5	-16.4	14.1 to 20.2	16.1
SSLA2BS	30 May 1993	M	SA	-16.0 to -15.4	-15.7	16.7 to 10.0	18.8
SSLA6BS	9 June 1993	M	SA	-15.6 to -14.1	-14.8	17.6 to 19.7	18.7
SSLB1BS	21 May 1994	M	SA	-17.4 to -15.0	-15.9	17.7 to 22.3	19.8
SSLB2BS	22 May 1994	M	A	-15.9 to -13.7	-15.0	16.5 to 20.5	18.3
SSLB3BS	20 May 1994	M	SA	-16.8 to -15.8	-16.3	15.1 to 17.0	16.0
SSLB4BS	23 May 1994	M	SA	-17.6 to -16.5	-17.1	15.1 to 16.8	15.7

Table 3.3 – cont.

Pinniped	Sample Date	Sex	Age	Range $\delta^{13}C$	$\bar{x}\ \delta^{13}C$	Range $\delta^{15}N$	$\bar{x}\ \delta^{15}N$
Steller sea lions - Pribilof Islands, Bering Sea – cont.							
SSLB5BS	23 May 1994	M	SA	-17.2 to -14.1	-16.0	15.3 to 20.5	17.3
SSLB6BS	24 May 1994	F	A	-15.3 to -14.2	-14.6	16.7 to 20.5	19.1
SSLB7BS	24 May 1994	M	SA	-17.2 to -14.0	-15.1	16.0 to 21.4	19.2
SSLB8BS	23 May 1994	F	SA	-16.8 to -15.5	-16.1	16.8 to 18.3	17.6
SSLB9BS	24 May 1994	M	A	-16.8 to -13.8	-15.4	14.8 to 19.8	16.8
SSLB10BS	24 May 1994	M	SA	-16.2 to -14.7	-15.5	17.1 to 21.4	19.1
SSLB11BS	25 May 1994	M	SA	-17.8 to -16.4	-17.0	14.7 to 17.1	15.5
SSLB12BS	30 Oct. 1994	M	P	-17.2 to -15.3	-16.4	15.9 to 18.1	17.3
SSLB13BS	2 Oct. 1994	F	SA	-18.1 to -15.8	-17.1	12.5 to 17.2	14.9
SSLB14BS	2 Oct. 1994	M	SA	-17.8 to -14.9	-16.7	14.5 to 18.4	16.2
SSLB15BS	28 May 1994	M	A	-16.9 to -14.0	-15.5	14.9 to 19.8	17.1
SSLB16BS	5 May 1994	M	U	-16.4 to -13.6	-15.0	15.6 to 20.1	18.5
SSLC1BS	26 Feb. 1995	M	A	-16.5 to -14.3	-15.2	17.3 to 18.5	18.0
SSLC2BS	10 May 1995	M	A	-15.8 to -14.4	-15.1	18.5 to 20.7	19.6
SSLD1BS	21 Jan. 1996	F	A	-16.1 to -14.6	-15.1	16.0 to 18.5	17.1
Steller sea lions - Chirikof Island, Gulf of Alaska							
SSLA0CH	12 June 1993	F	A	-16.8 to -14.1	-15.8	13.3 to 17.9	15.1
SSLA463WCH	14 June 1993	F	A	-17.5 to -15.0	-16.4	13.1 to 15.7	14.1
SSLA464WCH	14 June 1993	F	A	-15.3 to -13.5	-14.1	16.2 to 18.8	17.7
SSLA465WCH	15 June 1993	F	A	-16.9 to -14.9	-16.2	14.4 to 18.0	15.4
SSLA466WCH	15 June 1993	F	A	-17.4 to 15.8	-17.1	14.0 to 15.6	14.8
SSLA521WCH	17 June 1993	F	A	-16.2 to -13.2	-14.5	14.6 to 19.1	17.1
SSLA526WCH	11 June 1993	F	A	-17.4 to -15.2	-16.4	14.0 to 16.7	15.2

Table 3.3 – cont.

Pinniped	Sample Date	Sex	Age	Range $\delta^{13}C$	$\bar{x}$ $\delta^{13}C$	Range $\delta^{15}N$	$\bar{x}$ $\delta^{15}N$
Steller sea lions - Chirikof Island, Gulf of Alaska – cont.							
SSLA527WCH	11 June 1993	F	A	-17.5 to -15.5	-16.6	14.3 to 15.9	15.1
SSLA528WCH	12 June 1993	F	A	-15.6 to -12.4	-13.8	15.8 to 19.6	17.9
SSLA530WCH	13 June 1993	F	A	-16.9 to -14.3	-15.9	13.5 to 17.2	15.0

the Alaska Peninsula and Kodiak Island that would likely only be obtained during migration away from the Pribilof Islands. Small squid from waters around the Alaska Peninsula were at a trophic level consistent with the minimum $\delta^{15}N$ values found in the fur seal vibrissae.

Maximum isotope values along vibrissae from Chirikof Island Steller sea lions were consistent with a diet consisting of walleye pollock from the Alaska Peninsula and possibly the southern Bering Sea. The minimum isotope values in vibrissae from the GOA sea lions had no corresponding prey from the list of sampled organisms. The vibrissae from Bering Sea Steller sea lions had maximum isotope values consistent with a diet of gadids (both Pacific cod and large walleye pollock) from the Bering Sea, Alaska Peninsula and Kodiak. The minimum isotope values from the Bering Sea sea lions may indicate a diet of walleye pollock derived from waters along the Alaska Peninsula and near Kodiak Island.

DISCUSSION

Isotopic variability

A previous study by Hirons et al. (Chapter 2) revealed that Steller sea lions appear to grow and retain their vibrissae for several years. Sea lions in captivity that have had vibrissae removed or broken were observed rapidly replacing those vibrissae with new ones. Therefore, vibrissae from Steller sea lions in the northeast Pacific Ocean appeared to represent multiple years of growth and the oscillating

fluctuations in the stable isotope data in the vibrissae represent interannual cycles likely caused by trophic variability and/or geographic movement made by the species from year-to-year. Northern fur seal vibrissae also exhibited a regular oscillation in the carbon and nitrogen isotope ratios. Though no vibrissae growth data are currently available for this species, it can be argued that fur seals also retain their vibrissae for several years and the oscillations resulted from the annual migrations this species made from the Bering Sea into the North Pacific Ocean and back each year. The movements made by both species would result in foraging in various regions with different isotope ratios. Prey being consumed may also differ by region, further complicating the isotopic picture.

Isotope ratios in prey species

The overlapping data shown for prey isotope ratios from different geographic regions illustrates the difficulty of isotopically assessing diet in areas where heterogeneity exists in the surrounding isotopic environments. Depending upon the size and source area of the prey, the isotope ratios may vary by several parts per thousand. As the contour maps of Schell et al. (1998) illustrate, it is possible for a consumer to acquire a markedly different pattern of $\delta^{15}N$ and $\delta^{13}C$ by feeding in locations only a few hundred kilometers apart. Similarly, travel of prey species onshelf or offshelf will result in the prey having isotope ratios different from the source geographic region. Time for sufficient feeding to occur in the new region will result in a turnover of body carbon and nitrogen. For example, the stable isotope

values in the Bering Sea are more enriched onshelf and more depleted in the pelagic waters beyond the shelf. The comparison of the Bering Sea inner shelf and shelf break food web components showed a significant difference in the $\delta^{13}C$ of calanoid copepods, but that difference was not detectable in the upper trophic level organisms, which move across the isotopic boundaries seasonally or while feeding.

Components from the shelf break food web were compared with the Aleutian Shelf food web due to the proximity of the prey to pinniped haulouts in the Bering Sea, primarily the Pribilof Islands and rookeries in the Aleutian Islands. The isotopic differences between the adult herring of the Bering Sea and Aleutian Shelf may arise from the herring having feeding restricted to the region in which they resided. The adult herring were two trophic levels greater than copepods for their respective areas and that may indicate a significant fraction of ichthyoplankton in their diet. Juvenile herring from the Bering Sea were not available for comparison, but juveniles from along the Aleutian Shelf fit the trophic level between the Aleutian copepods and adult herring.

Benthic organisms, such as sole and octopus from the Bering Sea shelf break and sole and shrimp from the southern side of the Alaska Peninsula and around Kodiak Island, have enriched $\delta^{13}C$ values relative to $\delta^{15}N$. Arrowtooth flounder along the Aleutian Islands were almost 4‰ lower in $\delta^{15}N$ than those found around Kodiak Island but both had enriched carbon values. Fauna from benthic environments are characteristically more enriched than those from overlying waters as the lighter

isotope of detrital material is lost preferentially during decompositional respiration (Fry and Parker 1979). Detrital food webs, based on bacterial productivity, are typically enriched in $\delta^{13}C$ by 1‰ over the water column food web (France 1995).

Northern fur seals

The maximum isotope ratios in the vibrissae are consistent with a diet derived from walleye pollock from the western portion of the Gulf of Alaska and through the Bering Sea shelf break. Fur seals have been observed foraging in the summer in the passes between the islands for pollock, capelin and Atka mackeral. The few samples of Atka mackeral that Hobson et al. (1997) reported for the Gulf of Alaska are consistent with the maximum isotope values in the fur seal vibrissae; however, the depleted carbon values found in mackeral from the Bering Sea do not correspond to the fur seal vibrissae. The depleted $\delta^{15}N$ values exhibited in the vibrissae do not match any of the prey species sampled in the three study areas, but the northern region in the Gulf of Alaska was found to have low $\delta^{15}N$ values in the zooplankton (this study). Because of the large migration distances of this species, it is likely the minimum values exhibited by the fur seals correspond to prey from food webs farther south in the offshore pelagic waters of the Gulf of Alaska or in the North Pacific Ocean. Walleye pollock and flounder isotope values given by Hobson et al. (1997) for North Pacific species off the coast of Washington and Oregon, as well as pollock and squid values from other regions of the Gulf of Alaska, have the $\delta^{15}N$ values to produce the minimum isotope values observed in the vibrissae. Squid have also been

recorded as a prominent prey type in fur seal diets (Kajimura 1985; Perez and Bigg 1986; Sinclair et al. 1994). The isotope values for squid throughout the Bering Sea and Gulf of Alaska corresponded to those found in their vibrissae.

Steller sea lions

The stable isotope data appear to corroborate the natural history information gathered for the Bering Sea and Gulf of Alaska sea lions. Sea lion vibrissae sampled from Chirikof Island had the most enriched values and were likely the result of predominantly foraging on flatfish in the waters around Chirikof and Kodiak islands. The more depleted values may derive from pollock foraged in the waters around the Aleutian Islands and south of the Alaska Peninsula.

The isotope ratios for Bering Sea sea lions were similar to those expected from a diet of pollock from along the Alaska Peninsula. During late fall and winter, more sea lions are seen in the coastal waters and along the edge of the Bering Sea shelf. They may be feeding on large (>650mm) pollock, Pacific cod and sole in the deeper waters off the shelf break at this time and then move back to the rookeries in late spring.

Almost all of the animals showed variations in both $\delta^{13}C$ and $\delta^{15}N$ along the vibrissae and the magnitude of the changes appeared to be related, i.e. large oscillations in $\delta^{13}C$ were usually coincident with large oscillations in $\delta^{15}N$ (Figure 3.3). Such oscillations are also in phase but a shift in $\delta^{15}N$ alone could arise from a trophic change due to prey switching within a given environment. There is also a

possibility that movements across isotopic gradients may be accompanied by changes in dietary selection leading to increased or decreased trophic status with consequent shifts in $\delta^{15}N$. Steller sea lions in the Gulf of Alaska, however, showed these periodic oscillations most strongly and they are likely related to movement through geographic gradients on a seasonal basis (Figure 3.4). If the oscillating patterns show annual seasonal migrations between regions of isotopic difference, then some of the Bering Sea sea lions may be moving into specific regions and staying for time periods longer than a season. The $\delta^{13}C$ enrichment in Bering Sea sea lions may be an indication these animals were feeding more heavily on benthic animals, e.g. shrimp or flatfishes, in enriched coastal waters. That was evidenced by maximum isotope values in these sea lions most closely matching the isotope ratios of rock and yellowfin sole found in the Bering Sea, along the Alaska Peninsula and around Kodiak Island.

Stable isotope ratios are consistent with the presence of squid and juvenile walleye pollock in northern fur seals. Only some Steller sea lions isotope ratios corresponded to Pacific cod and pollock from the Bering Sea and western GOA and some Atka mackeral from along the peninsula. Seasonal prey types, such the salmon species, have not been addressed in our limited food webs. These species are expected to affect the isotope ratios in the pinniped tissues if eaten, but the isotope ratios of the salmon may be masked by similar trophic level prey.

Specific feeding locations and strategies remain largely unknown for these seals and sea lions. Satellite telemetry is now able to provide information on the

physical features of the water in which the animals are foraging as well as dive depth and duration. These physical characteristics, such as water temperature, assist in determining which prey choices may be residing at a particular depth and temperature (Merrick et al. 1997). In studies of trophic dynamics, stable isotope ratios can provide information on the trophic level of organisms and composition within a food web. As we have seen in this study, the combination of stable isotope analysis with pinniped ecology has provided further information on the animals' food web dynamics. With all this data, we could still only identify at what trophic level the animal was feeding and perhaps a generalized location. Stable isotope analysis, used in combination with satellite-linked time-depth recorders, biology and natural history, has the ability to provide greater information on foraging locations and be more species-specific when analyzing the diets of seals and sea lions.

ACKNOWLEDGMENTS

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CHAPTER 4

TROPHIC VARIABILITY OF HARBOR SEALS (*PHOCA VITULINA*) IN PRINCE WILLIAM SOUND, ALASKA

ABSTRACT

The number of harbor seals (*Phoca vitulina*) in Prince William Sound, Alaska has declined to approximately one-fourth of the 1975 population and food web dynamics have been investigated as a possible factor in the decline. Archived and recent harbor seal tissues were used to determine food web structure and trophic dynamics of seals within Prince William Sound and the adjacent Gulf of Alaska. Within the sound, carbon and nitrogen isotope ratios confirm that most harbor seals are near the top of food chains that are based on local, in situ primary and secondary productivity and not on production from outside the sound. Carbon isotope ratios also indicated that benthic prey are a large component of harbor seal diets. Isotope ratios along wild seal whiskers show that some individuals migrate into areas (presumably in the Gulf) wherein food web structures are different and isotope ratios of prey are considerably lower than within the sound. Data on isotope ratios of potential prey species from Prince William Sound and from other sites in the Gulf of Alaska indicate a geographic isotopic gradient exists between onshelf and deep pelagic waters.

Hirons AC, Schell DM, Kline TC (in prep) Trophic variability of harbor seals (*Phoca vitulina*) in Prince William Sound, Alaska. Mar Mamm Sci

INTRODUCTION

Severe declines in harbor seal (*Phoca vitulina*) populations in Prince William Sound and the Gulf of Alaska have been observed for more than two decades. The population of harbor seals in Prince William Sound (PWS) was further impacted by the *Exxon Valdez* oil spill in 1989 and the population has continued to decline by 6% per year (Frost et al. 1999)..Speculation regarding the role that prey availability has had on these declines has led to the use of stable isotope ratios in assessing food web and trophic linkages. Stable carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) are established in the primary producers of the food chain and act as natural tracers of carbon and nitrogen transfers through the food webs. This in turn may provide evidence of prey consumed at different locations and trophic levels within PWS and the adjacent Gulf of Alaska (GOA).

Carbon isotope ratios ($^{13}C/^{12}C$) serve as relatively conservative, within 1‰, tracers of energy supply among trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc. acquire the isotope ratios in proportion to the amount of food derived from each differing source (DeNiro and Epstein 1978, Fry and Sherr 1984). This, in turn, is reflected in the composition of body tissues and in keratinous tissues, e.g. baleen and whiskers, as a temporal record when differing sources of food are consumed over time and space (Schell and Saupe 1993). This allows us to discern important habitats and food resources in animals that seasonally migrate or shift diets.

Nitrogen isotope ratios ($^{15}N/^{14}N$) reflect both the food sources and the trophic status of the consumer. As nitrogen in food is consumed and assimilated by an animal, the heavy isotope is retained and the animal is enriched by approximately 3‰ with accompanying loss of the lighter isotope through excretion (DeNiro and Epstein 1981, Minigawa and Wada 1984). The isotopic composition of muscle tissue in an organism tends to reflect the isotopic makeup of the entire organism and, therefore, fractionation differences among tissues can be determined by comparing the isotope ratios of various tissues against those of muscle (DeNiro and Epstein 1978, 1981). Enrichment occurs with each trophic step and allows the construction of conceptual models and food webs and the assignment of trophic status to species for which dietary data are sparse. The data obtained from these measurements are unique in that they trace materials actually assimilated and can thus be used for ecosystem modeling.

The objectives of this isotope study were to collect and analyze tissues from harbor seals and prey species in the vicinity of PWS and the GOA for purposes of assessing if dietary changes had occurred and might have contributed to their population declines. It can be hypothesized that the natural stable isotope abundances of PWS biota will shift in response to changes in trophic level, food web structure, and primary productivity in the environment, thus providing an independent tool to verify, quantify, and model ecosystem processes. The isotopic tracer approach enables the integration of ecosystem components.

METHODS

Through the use of carbon isotope data on taxa collected over geographical regions, the presence/absence of isotopic gradients useful in sorting out habitat dependencies were determined. Nitrogen isotope ratios were used to assign trophic status to species in each region. Temporal changes in harbor seal trophic status and food dependencies were determined by comparing isotope ratios along the lengths of vibrissae with isotope ratios from available prey.

Prey species

Stable isotope values of zooplankton from Prince William Sound and the Gulf of Alaska were obtained from literature sources and databases ranging from 1994 to 1997 (Schell et al. 1998; Kline 1999). These data were used to test for geographic variations that would help identify food webs and potential movement in harbor seals. In addition, lower trophic level organisms, consisting of both pelagic and benthic species residing within and outside PWS, were obtained and analyzed between 1993 and 1997. Personnel from the Alaska Department of Fish and Game, the National Marine Fisheries Service and the Prince William Sound Science Center assisted in the collection of these organisms. A few grams of muscle tissue were extracted from several samples of each species from a location. The tissues were frozen in a standard -10°C freezer and transported to the stable isotope facility at the University of Alaska Fairbanks for analysis.

Pinnipeds

Harbor seal tissues were collected with the assistance of the Alaska Department of Fish and Game (ADFG) and native subsistence hunters. From 1993-1998 vibrissae and muscle tissue from 128 harbor seals were analyzed for stable isotope ratios. Additional vibrissae from live harbor seals were collected within PWS and from the surrounding GOA (Figure 4.1). One to two long vibrissae were pulled from live animals being monitored by ADFG or from harvested animals for stable isotope analysis. Vibrissae were scrubbed with steel wool to remove any debris and segmented from base to tip in 2.5mm segments. Every other segment was analyzed for carbon and nitrogen isotope ratios and the reserved segments were archived for future reference. When possible, samples of muscle tissue were taken for analysis. Approximately one cubic centimeter of muscle tissue was collected from dead animals. The tissues were kept frozen between the time of collection and isotope analysis. The isotopic ratios of vibrissae and muscle tissue from 14 harbor seals were compared to determine fractionation differences between vibrissae and muscle tissues.

Analytical Techniques

All prey and harbor seal muscle tissue samples were dried in a laboratory oven at 60°C for a minimum of forty-eight hours and ground for homogeneity while vibrissae segments were analyzed without additional preparation. The isotope ratios of carbon and nitrogen were determined with a Europa 20/20 continuous flow isotope ratio mass spectrometer. The samples were combusted at high temperature and the

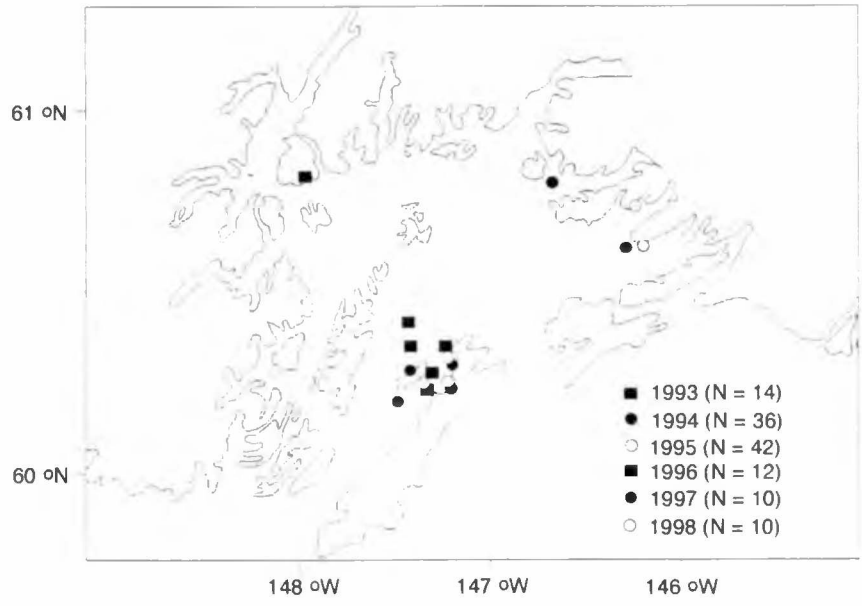

Figure 4.1. Sample locations for harbor seals in Prince William Sound, 1993-1998.

nitrogen and carbon dioxide gases separated and purified by gas chromatography. The gases were subsequently led into the mass spectrometer by capillaries and the isotope ratios determined. All samples were analyzed in duplicate. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation relative to Pee Dee Belemnite and air standards for carbon and nitrogen, respective of the standards. Standard replicates were analyzed for every twelve samples. If the difference between replicates was greater than 0.5‰, samples were reanalyzed. A difference of 0.2‰ was considered acceptable. Analytical error for samples was approximately ±0.1‰ for both carbon and nitrogen.

Statistical Analysis

Hotelling's T-test was used to distinguish if regional differences existed among harbor seals based on their stable isotope ratios. Multiple analyses of variance (MANOVA) and Wilk's Lambda were used to investigate isotopic differences based on the sex and age of the seals and the region of PWS and the years that samples were gathered. Fractionation differences in the harbor seal tissues were calculated using least square means and standard error equations. (SYSTAT 1997).

RESULTS

Isotope Ratios in Prey Species

The isotope ratios of prey species important to harbor seals were defined within and outside PWS. The prey plots (Figures. 4.2 and 4.3) were created using $\delta^{13}C$ and $\delta^{15}N$ values for nine potential prey species for harbor seals. *Neocalanus* spp. were

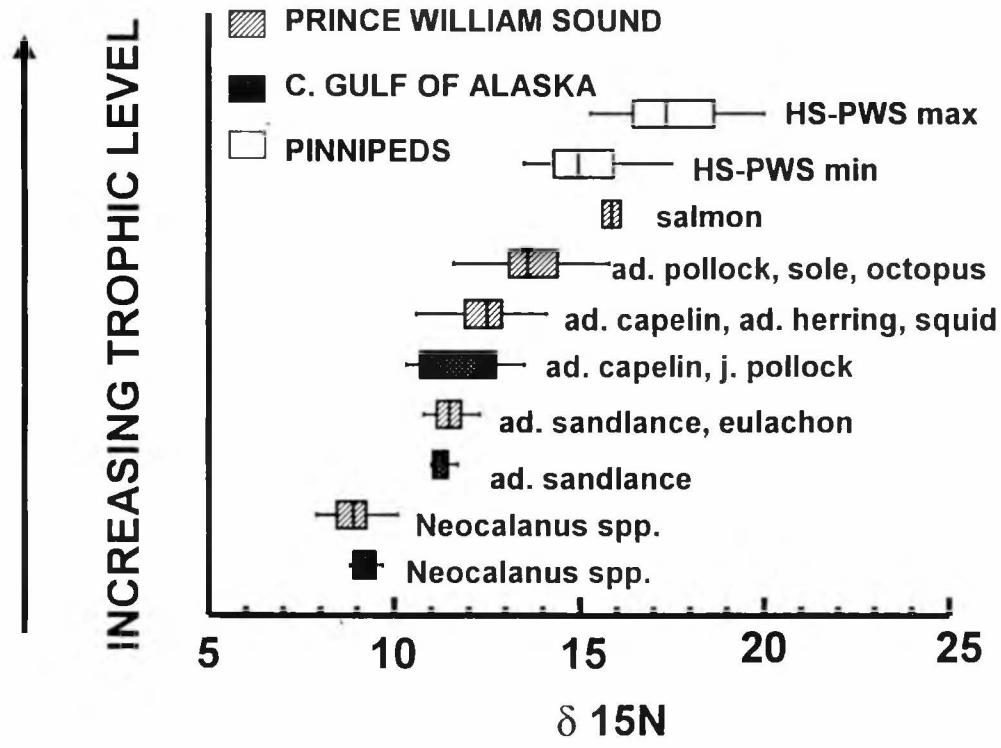

Figure 4.2. Maximum (max) and minimum (min) mean (± SE) $\delta^{15}N$ from Prince William Sound harbor seals (PWS HS) and mean (± SE) $\delta^{15}N$ from Prince William Sound and Gulf of Alaska fishes and invertebrates. Vibrissae values have been normalized to muscle. Sample sizes are ≥ 5. Species with similar isotope ratios were grouped for clarity.

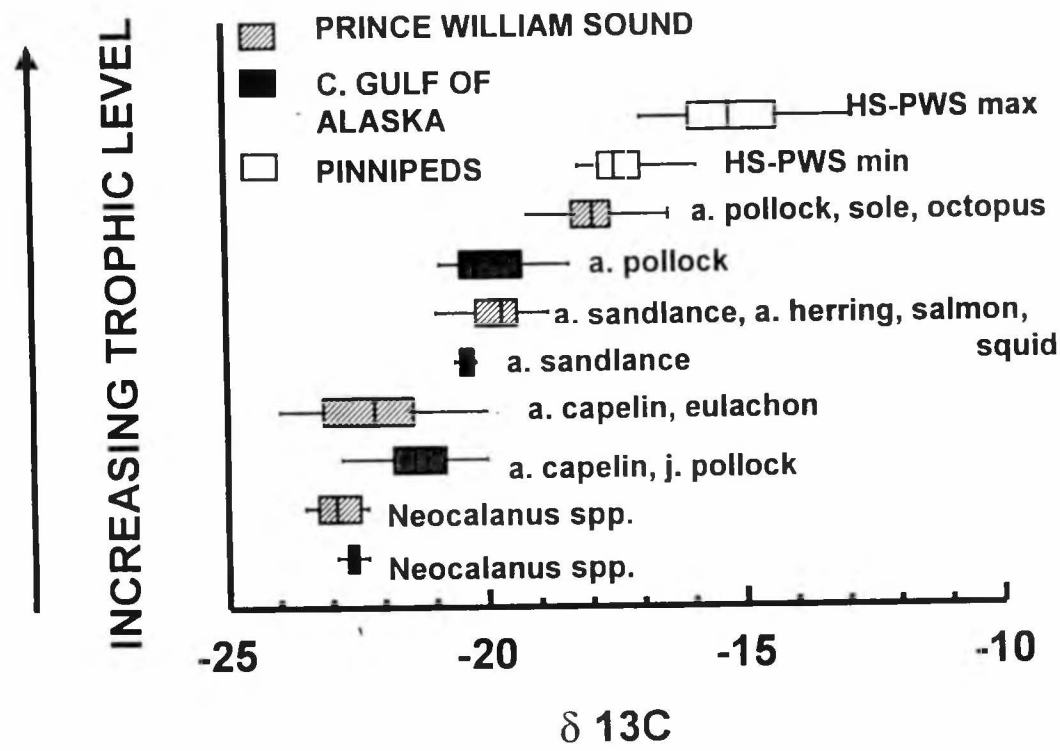

Figure 4.3. Maximum (max) and minimum (min) mean (± SE) $\delta^{13}C$ from Prince William Sound harbor seals (PWS HS) and mean (± SE) $\delta^{13}C$ from Prince William Sound and Gulf of Alaska fishes and invertebrates. Vibrissae values have been normalized to muscle. Sample sizes are ≥ 5. Species with similar isotope ratios were grouped for clarity.

included in the food web as a first-order consumer within the sound. For the sake of clarity, only a random sampling of harbor seals was added to the plot. These plots are not meant to represent the absolute prey variety in the diet, but more as likely sources of prey for seals foraging within the sound.

Isotope Ratio Variations in Wild Harbor Seals

Using least square means, harbor seal vibrissae were enriched by 1.9‰ in $\delta^{13}C$ relative to muscle while the vibrissae were only slightly enriched by 0.1‰ in $\delta^{15}N$ relative to muscle. These results allowed the vibrissae values to be normalized to muscle values so a direct comparison of isotope values could be made to muscle from prey organisms.

Seals from 11 sites were sampled in PWS (Figure 4.1). The $\delta^{13}C$ and $\delta^{15}N$ values at the base of the vibrissae were used for statistical analysis. The isotope values at the base of the vibrissae reflect the prey most recently incorporated by the animals and the likelihood is greater that the prey were found in the area where the seals were sampled. Nine of the 11 subregions within PWS were in close proximity to one another and were grouped for analysis. The two remaining locations in northeastern Prince William Sound were grouped together for analysis. Adult and subadult harbor seals from the 9 areas in southern PWS were significantly different from the two northeastern sites in PWS by area, $F_{16,1896} = 19.1$, $p = <0.001$. The seals were also significantly different by sex $F_{2,955} = 11.1$, $p = <0.001$ and age $F_{4,1908} = 45,953$, $p = <0.001$. The two areas in northeastern PWS were significantly different from each

other $F_{2,86} = 11.8$, $p = <0.001$. There is a significant difference in age $F_{4,170} = 9.4$, $p = <0.001$ but not between sexes.

Stable isotope ratios within harbor seal vibrissae do not appear to fluctuate greatly or with any periodicity, although some seals do show large changes between enriched and depleted values. Harbor seals sampled in 1993 had relatively constant $\delta^{13}C$ values and some fluctuations (<2‰) in $\delta^{15}N$ values that likely corresponded to seasonal changes in primary prey type. The periodicity of the fluctuations in the 9 seals does not appear regular. Six of 10 seals sampled from southern PWS in the spring of 1994 had large, synchronous fluctuations in $\delta^{13}C$ and $\delta^{15}N$, as large as 5.5‰ (Figure 4.4). Two-thirds of the seals sampled in September 1994 had synchronous fluctuations larger than 1‰ in $\delta^{13}C$ and $\delta^{15}N$ in at least one location along the length of the whisker. Six of the 12 whiskers analyzed in spring of 1995 and 6 out of 7 in fall of 1995 also had synchronous fluctuations larger than 1‰ in $\delta^{13}C$ and $\delta^{15}N$ in at least one location along the length of the whisker. A random sampling of seals in the spring and fall 1996 as well as summer 1997 and 1998 revealed that a majority of the animals had fluctuations greater than 1‰ in $\delta^{13}C$ and $\delta^{15}N$. The isotope fluctuations in the seal vibrissae were separated into maximum and minimum values based on differences greater than 1‰ in $\delta^{13}C$ and 3‰ in $\delta^{15}N$, respectively. Vibrissae with fluctuations in $\delta^{13}C$ and $\delta^{15}N$ less than 1‰ and 3‰ had their isotope values averaged for the entire whisker.

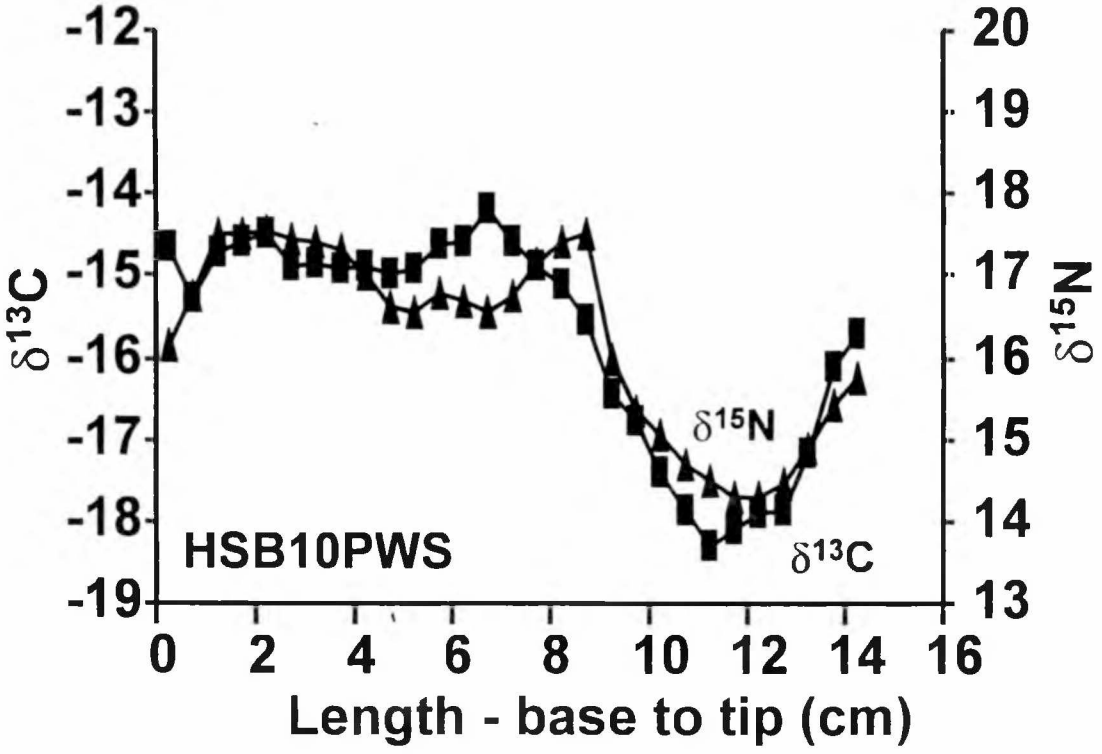

Figure 4.4. $\delta^{13}C$ and $\delta^{15}N$ in a harbor seal vibrissa from Prince William Sound, Alaska, May 1994. The base of the vibrissa begins at 0cm.

DISCUSSION

The isotope ratio gradients in selected zooplankton species were first identified in the waters of the Beaufort and Chukchi seas and were further defined for the Bering Sea and published by Saupe et al. (1989) and Schell et al. (1998). The pronounced isotope ratio gradients observed in Bering Sea zooplankton led to the belief that similar gradients might be present in the Gulf of Alaska and extending into Prince William Sound. Knowledge of the magnitude and position of these gradients was essential for the interpretation of observed shifts in isotope ratios in seal vibrissae. The presence of a geographic gradient in carbon and nitrogen isotope ratios declining with distance offshore has been shown to exist along the Gulf of Alaska coast. Kline (1999) found an isotopic gradient between *Neocalanus cristatus* from the northern Gulf of Alaska just south of Prince William Sound and *N. cristatus* within the Sound. An approximate 4‰ depletion exists in $\delta^{13}C$ of the calanoid copepods outside the sound relative to those within the sound (Kline 1997). Similar isotopic gradients of 2‰ have been identified by Schell et al. (1998) for zooplankton in the Bering Sea and Aleutian Islands. They found on-shelf and shelf-break waters more enriched and deep-water regions more depleted in $\delta^{13}C$ and $\delta^{15}N$. Zooplankton data (this study) (Fig. 4.5 and 4.6) and Kline (1999) both show that the Gulf of Alaska has markedly lower isotope ratios for both carbon and nitrogen in offshore waters versus those found within the sound. Hobson et al. (1994) found onshore-offshore differences in their study of seabirds in the GOA. Perry et al (1999) also reported cross-shelf depletion in carbon

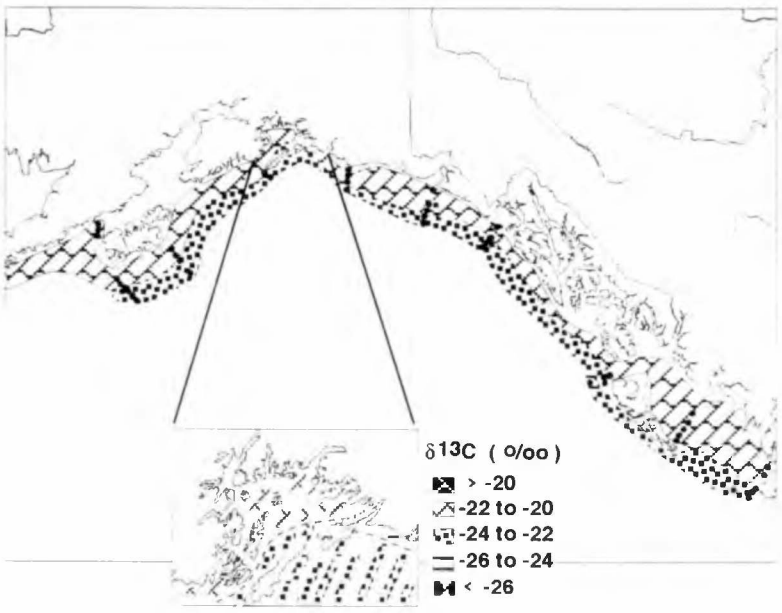

Figure 4.5. $\delta^{13}C$ isotope contours for calanoid copepods in the Gulf of Alaska and Prince William Sound.

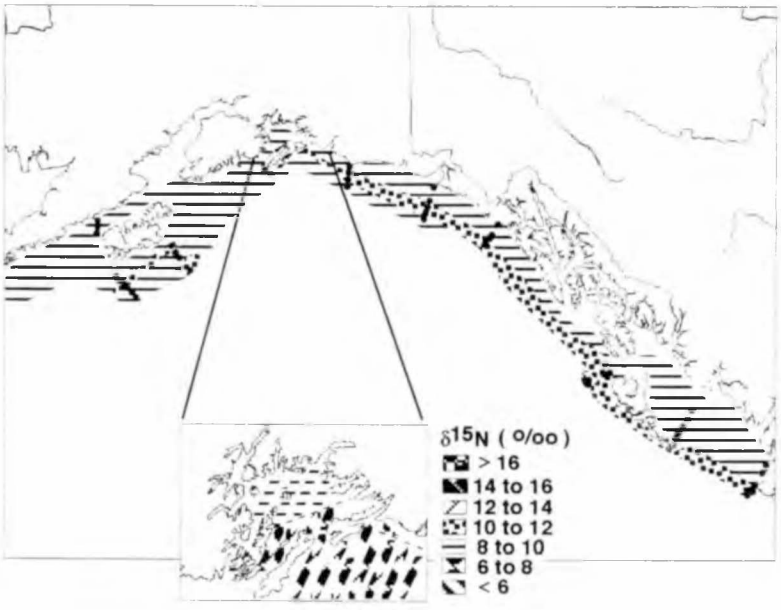

Figure 4.6. $\delta^{15}N$ isotope contours for calanoid copepods in the Gulf of Alaska and Prince William Sound.

isotopes from shelf to slope water off the coast of British Columbia, Canada. Although data are not available for the central GOA, indications are that both nitrogen and carbon isotope ratios reach a minima within some distance offshore.

A previous study by Hirons et al. (Chapter 2) revealed that harbor seals appear to grow their vibrissae for several months during a year and then replace them every year. The start of the replacement appeared to be during the summer months when breeding and molting occurred. New vibrissae growth continued into winter and ceased by late spring. Additionally, combative male harbor seals that had broken vibrissae were observed rapidly replacing those vibrissae with new ones (Bowen per comm.) Therefore, harbor seal vibrissae from the northeast Pacific Ocean appear to represent one year's growth or less. There is no interannual variability in the stable isotope data in the vibrissae so whatever trophic or geographic changes which resulted in the isotopic fluctuations occurred during the span of a year.

The stable isotope differences observed in the vibrissae of seals from different locations appeared to agree with some of the location differences defined by the fatty-acid analysis for harbor seals in the sound (Iverson et al. 1997). For example, Iverson et al. suggested flatfish were eaten by seals west of Montague Island in PWS. These differences may have resulted from juveniles of a prey species being eaten in one region of the sound while adults of the same species were eaten in another region. Frost et al. (1998) reported observing different foraging patterns in adult and subadult seals as well as differences between males and females at various locations within and

outside the sound throughout the year. These differences may explain the isotopic variations observed in the seal vibrissae.

Based on the natural history of harbor seals, including information from stomach content analyses, pollock, herring, squid, octopus, salmon, and capelin were evident most often in seal stomachs from Prince William Sound (Pitcher 1980). The pleuronectid, yellowfin sole, had been observed being taken by seals in an area west of Montague Island during this study so a few samples of these, as well as high-lipid eulachon, were collected and added to the plot. Prey data, e.g. from herring and pollock, have shown very little isotopic variation among locations within the sound. However, prey data collected south of PWS during the 1996 National Marine Fisheries Service Gulf of Alaska survey revealed some evidence of an isotopic gradient in higher trophic organisms. The $\delta^{13}C$ data for adult pollock revealed an average enrichment of 2‰ compared to those sampled from PWS than those found along the shelf south of the sound (Figure 4.3). A similar enrichment was also found in the $\delta^{15}N$ of pollock from sound versus those found in the offshore waters (Figure 4.2).

Based on historical information, harbor seals appear to forage on one to two preferred prey but will also feed on seasonally available species such as salmon (Pitcher 1980; Iverson et al. 1997). The $\delta^{15}N$ values in harbor seals having the more enriched stable isotopes ("max") (mean $\delta^{15}N = 17.2$) were isotopically similar to those from pollock, yellowfin sole, octopus, and silver salmon from PWS, based on a 3‰ trophic level enrichment in marine food webs (DeNiro and Epstein 1981; Schoeninger

and DeNiro 1984, Hobson et al. 1994) (Figure 4.2). However, the "max" $\delta^{13}C$ in the seals (mean $\delta^{13}C$ = -15.4) was even more enriched than the expected 1‰ trophic increase above any of the prey species sampled either in PWS or in the GOA (DeNiro and Epstein 1978; France and Peters 1997) (Figure 4.3). The $\delta^{15}N$ values in harbor seals having the more depleted stable isotopes ("min") (mean $\delta^{15}N$ = 14.7) were isotopically similar to those from capelin and pollock from the Gulf of Alaska south of the sound, and capelin, herring, and squid found within the sound (Figure 4.2). The $\delta^{13}C$ values in these seals ("min") (mean $\delta^{13}C$ = -17.6) were most similar to those in pollock from the GOA and in herring, sandlance and squid from PWS (Figure 4.3).

Hobson et al. (1997) reported harbor seals from the Copper River Delta (CRD) in the Gulf of Alaska having mean $\delta^{15}N$ = 18.6 and mean $\delta^{13}C$ = -17.6. These nitrogen values were more enriched than any found in seals residing in PWS. As Hobson et al. pointed out, the seals from the CRD were likely sampled at a time when they were foraging on returning coho (silver) salmon, which could have accounted for the high nitrogen values. The carbon values for the CRD seals were very similar to the "min" values for PWS seals and provided additional evidence supporting the hypothesis that the depleted $\delta^{13}C$ values in some PWS seals resulted from foraging on prey outside the sound. Because depleted isotope values were expected in pelagic food webs, the enriched values along the vibrissae were assumed to correspond to prey from the sound while the depleted values corresponded to prey from the Gulf of Alaska. Because harbor seals tend to have strong site fidelity, it is thought that seals

with constant isotope ratios foraged near their haul-out sites in Prince William Sound (Pitcher and McAllister 1981). The decrease in isotope ratios with distance offshore appeared evident in the vibrissae in a number of individual harbor seals, indicating movements into offshore feeding areas during a part of the annual cycle represented in a vibrissa. These low values provided a distinctive geographic indicator visible in vibrissae of seals that fed in pelagic regions or on prey that emigrated from offshore areas.

These isotope data are also consistent with satellite tag data that showed relatively limited movements by most harbor seals but occasional extensive movements into the Gulf of Alaska by some individuals. Data from satellite tagged harbor seals have provided evidence that some seals of all ages traveled outside PWS; in some cases, seals have been tracked traveling more than 100 km into the Gulf of Alaska. The number of seals and amount of movement by seals tagged during 1992-1996 varied considerably and that variability seemed to be represented in the vibrissae. A greater number of seals with isotopic fluctuations were observed from 1995-1998 than in the previous two years and that corresponded to the increased number of tagged seals observed leaving the sound by Frost et al. (1998). Vibrissae were collected from harbor seals for this study at the same time satellite tags were being deployed on seals. Unfortunately, data from the vibrissae would only represent the feeding during prior year while the satellite data provided information on seal movement for the following

year. Therefore, there were no overlapping isotope and satellite data for the seals in this study to confirm if a seal that traveled one year also traveled the next.

A conceptual model of harbor seal feeding has been constructed based on the known isotope ratios in lower trophic levels and fishes, primarily capelin, herring and pollock. Predicted isotope ratios in seals using these food sources matched observed $\delta^{15}N$ values closely, but the measured $\delta^{13}C$ values were generally higher than predicted. We suggest that benthos, which are usually enriched relative to pelagic species at a given site, are important in the food supply of these seals. Consumption of demersal or benthic organisms would be reflected in increased $\delta^{13}C$ values. Benthic environments tend to have more enriched values due to recycling of nutrients and the presence of bacterial food webs (Coffin et al. 1994, France 1995). Both yellowfin sole and octopus are benthic feeders, which would result in these organisms having more enriched $\delta^{13}C$. Seals feeding on these animals would exhibit those enriched values (Wells 1978).

The fluctuations indicate that the seals are relying upon more than one food web, shifting between pelagic vs. benthic or Prince William Sound vs. Gulf of Alaska. The cause of these isotopic fluctuations is not currently known, but we hypothesized that prey outside Prince William Sound were more depleted in stable isotopes and that some seals may have been foraging on the ^{13}C-depleted prey. With the data available, we are uncertain as to the definitive causes for these fluctuations.

The harbor seal population in Prince William Sound continues to be monitored by the Alaska Department of Fish and Game as part of a long-term research study. As part of that program, satellite tag data continues to be collected which could be useful in tracking the seals' movements as well as feeding habits. As more seals are tagged with satellite tracking units, the likelihood of recapturing seals and collecting vibrissae from them is greater. The stable isotope data gathered from these vibrissae could be combined with the movement information on the seals to provide a clearer picture of where and on what the seals have been feeding. This picture can be further enhanced with the use of fatty-acid analysis in more accurately identifying which prey the seals have been consuming.

ACKNOWLEDGMENTS

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CHAPTER 5

TEMPORAL RECORDS OF $\delta^{13}C$ AND $\delta^{15}N$ IN NORTH PACIFIC PINNIPEDS: INFERENCES REGARDING ENVIRONMENTAL CHANGE AND DIET

ABSTRACT

Sea lion and seal populations in Alaskan waters have undergone various degrees of decline during the latter half of the twentieth century and often the cause(s) for the declines remain uncertain. The stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios in bone collagen from wild Steller sea lions *(Eumetopias jubatus)*, northern fur seals *(Callorhinus ursinus)* and harbor seals *(Phoca vitulina)* from the Bering Sea and Gulf of Alaska were measured for the period 1951-1997 to test the hypothesis that a change in trophic level may have occurred during this interval and contributed to the population declines. A significant change in $\delta^{15}N$ in pinniped tissues over time would imply a marked change in trophic level. No significant change in bone collagen $\delta^{15}N$ was found for any of the three species during the past forty-seven years in either the Bering Sea or the Gulf of Alaska. However, the ^{15}N in the Steller sea lion collagen was significantly higher than both northern fur seals and harbor seals. A

Hirons AC, Schell DM, Finney BP (in press) Temporal records of $\delta^{13}C$ and $\delta^{15}N$ in North Pacific pinnipeds: inferences regarding environmental change and diet. Oecologia

significant decline in $\delta^{13}C$ (almost 2 ‰ over the 47 years) was evident in Steller sea lions, while a declining trend, though not significant, was evident in harbor seals and northern fur seals. Changes in foraging location, in combination with a trophic shift, may offer one possible explanation. Nevertheless, a decrease in $\delta^{13}C$ over time with no accompanying change in $\delta^{15}N$ suggests an environmental change affecting the base of the foodweb rather than a trophic level change due to prey switching. A decline in the seasonal primary production in the region, possibly resulting from decreased phytoplankton growth rates, would exhibit itself as a decline in $\delta^{13}C$. Declining production could be an indication of a reduced carrying capacity in the North Pacific Ocean. Sufficient quantities of optimal prey species may have fallen below threshold sustaining densities for these pinnipeds, particularly for yearlings and subadults who have not yet developed adequate foraging skills.

INTRODUCTION

Populations of Steller sea lions, northern fur seals and harbor seals have drastically declined for more than two decades, particularly in the western Gulf of Alaska and Bering Sea (Pitcher 1990; Loughlin 1993; ADFG 1996; Strick et al. 1997). These pinnipeds are generally found in coastal waters and along the continental shelf throughout the North Pacific Ocean, including the Bering Sea and the Gulf of Alaska (NRC 1996). Food limitation has been hypothesized as a likely cause behind the declines in the pinniped populations, potentially resulting from decreases in clupeid

fishes and increases in gadid fishes (Merrick et al. 1987; Alverson 1991; Trites 1992; Alaska Sea Grant 1993; Merrick 1995; Anderson et al. 1997; Merrick et al. 1997). In this paper we explore how the changes in physical and biological characteristics of this region may have impacted these animals.

Physical and biological changes in the northeast Pacific Ocean and Bering Sea

An abrupt climatic change occurred in the Pacific Ocean in the mid-1970s and a new "regime" continued through the 1980s (Hare and Mantua 2000). This change in atmospheric circulation altered wind patterns and intensity, mixed layer depth, sea surface temperatures, ice extent and depth of ocean current patterns (Royer 1989; Trenberth and Hurrell 1994; Freeland et al. 1997). However, little is known about how the effects of the changing environmental conditions influenced pinnipeds in the North Pacific Ocean.

The biological responses to these physical changes have manifested themselves in fluctuating phytoplankton abundance, changing zooplankton production and shifting migration patterns and biomass of commercial and non-commercial organisms (Venrick et al. 1987; Ebbesmeyer et al. 1991; Brodeur and Ware 1992; Hollowed and Wooster 1992; Francis and Hare 1994; Polovina et al. 1994; Hollowed and Wooster 1995; Polovina et al. 1995; Quinn and Niebauer 1995; Anderson et al. 1997; Anderson and Piatt 1999). Sugimoto and Tadokoro (1997) reported declining chlorophyll concentrations and zooplankton biomass during the mid-1970s and late 1980s in the eastern Pacific and Bering Sea, while peaks in both chlorophyll and zooplankton

occurred in the late 1960s and continued to decline after that point in the central Pacific.

Many forage fish stocks have dramatically increased while others have declined in the Gulf of Alaska and Bering Sea since the mid-1970s. The forage species composition for the region has shifted from an environment dominated by clupeid fishes and panaeid shrimp to one currently dominated by gadids and pleuronectids (Anderson et al. 1997; Anderson and Piatt 1998). In 1951 and 1964, samples from Steller sea lion stomachs from the Bering Sea showed that walleye pollock was the fourth most prevalent prey species (Fiscus and Baines 1966), but by 1976 pollock was the dominant prey (Lowry 1982; Lowry et al. 1989). Stomach content analyses of Pribilof Island fur seals in the early 1980s showed a predominance of juvenile walleye pollock and squid. Pacific herring and capelin, previously considered important prey, were absent (Sinclair et al. 1994). Kenyon (1965) noted that harbor seals from Amchitka Island in the Aleutian Archipelago had remains of octopus and Atka mackerel in their stomachs, whereas harbor seals sampled in 1979 from the Alaska Peninsula had primarily walleye pollock and octopus in their stomachs (Pitcher 1980).

Isotope ratios in food webs

The isotopic ratios of animal tissues, particularly in marine organisms, are slightly more enriched in ^{13}C (0.5 to 1‰) and ^{15}N (3 to 5‰) than those found in their diet (Hobson and Welch 1992; France and Peters 1997). Isotopic variations observed in organisms throughout the marine environment are believed to result from

differences that can originate at the base of food webs and metabolic pathways in the organisms (DeNiro and Epstein 1978, 1981; McConnaughey and McRoy 1979; Rau et al. 1983; Fry and Sherr 1984; Minigawa and Wada 1984; Sholto-Douglas et al. 1991; Hobson and Welch 1992; France and Peters 1997). Herbivorous zooplankton, consisting primarily of calanoid copepods and euphausiids in the North Pacific Ocean, are first-order consumers. Any changes affecting the stable isotope ratios within the phytoplankton, such as carbon and nutrient sources and their growth rate, would be carried through the food web and be reflected in foraging pinnipeds. Recent studies have shown a close correlation between cellular growth rates and carbon isotope ratios ($\delta^{13}C$) in phytoplankton. Rapid growth rates in phytoplankton result in reduced fractionation of the carbon being utilized and result in higher $\delta^{13}C$ values if other factors (CO_2, nutrients) remain constant. Laws et al. (1995) have shown a strong relationship between diatom growth rates and isotopic fractionation in the laboratory. Bidigare et al. (1997) confirmed these findings in laboratory studies of cultures of haptophyte algae and in phytoplankton sampled from various world ocean environments; increased growth rate and productivity in both the diatoms and haptophytes were correlated with increased $\delta^{13}C$ values.

Bone collagen is a tissue that has a relatively slow turnover rate, as much as ten years in large adult mammals. Depending on the age of the animal ($\leq$10 years), the stable isotope ratios in the collagen is likely integrated over much of its life (Hobson and Clark 1992; Ambrose and Norr 1993). This tissue acts as a long-term integrator of

isotope ratios and moderator of sporadic isotopic fluctuations, a factor that is useful when comparing isotope ratios of many individuals over long periods of time (Schoeninger and DeNiro 1984; Lee-Thorp et al. 1989). Episodic or short-term changes in dietary isotope ratios are dampened in the collagen record, leaving only changes in the long-term trends as an indicator of the organism's trophic status in its environment.

Stable isotope analysis of archived samples of bone collagen is a potential tool that may reveal processes associated with the recent declines in pinniped populations. Herring and capelin, once dominant in the diets of these pinnipeds, are generally of a lower trophic level than the walleye pollock currently being eaten by these seals and sea lions (this study). Thus, we hypothesize that this dietary change should be reflected as changes in bone $\delta^{13}C$ and $\delta^{15}N$ values. In addition, if changes in physical parameters altered primary production, this may be reflected in bone $\delta^{13}C$ values. As primary production regulates the carrying capacity for the entire food web, such changes could have important implications for top consumers such as seals and sea lions. If prey availability falls below threshold densities, recruitment would be greatly reduced.

METHODS

Pinniped samples

Seal and sea lion bone samples were obtained from native-harvested animals and museum skeletal collections at the University of Alaska Museum and the Kodiak

Historical Society. A total of 31 Steller sea lions, 13 northern fur seals and 63 harbor seals from regions throughout the Gulf of Alaska and the Bering Sea were analyzed. The specimens span the period of 1951-1997 and range from coastal areas of southeast Alaska westward through the Gulf of Alaska and into the central Bering Sea (Figure 5.1). The Gulf of Alaska was separated into three regions, based on distinct pinniped populations, for statistical analyses. The western Gulf of Alaska is defined as the area between 152°W and 175°W, the central Gulf of Alaska is the area between 144°W and 152°W and the southeastern Gulf of Alaska is the area between 130°W and 144°W.

Sex and age of the animal were not recorded for most of the specimens, though one sea lion skeleton was suspected as being from a pup on the basis of its size and dentition. The remaining skeletons were either labeled as adults or subadults; age unknown, or in some cases age group as estimated by dentition development. The data were not sufficient to test for age-related trophic differences. More than half of the sampled sea lions (61%) and northern fur seals (62%) came from the Bering Sea, and the remaining skeletal samples came from the western and central portions of the Gulf of Alaska. Harbor seal samples were evenly distributed among the Bering Sea, western, central and southeastern Gulf of Alaska. Samples for both the sea lions and the harbor seals were generally evenly distributed throughout the forty-seven year study period, but there were years when, at a minimum, no samples were available,

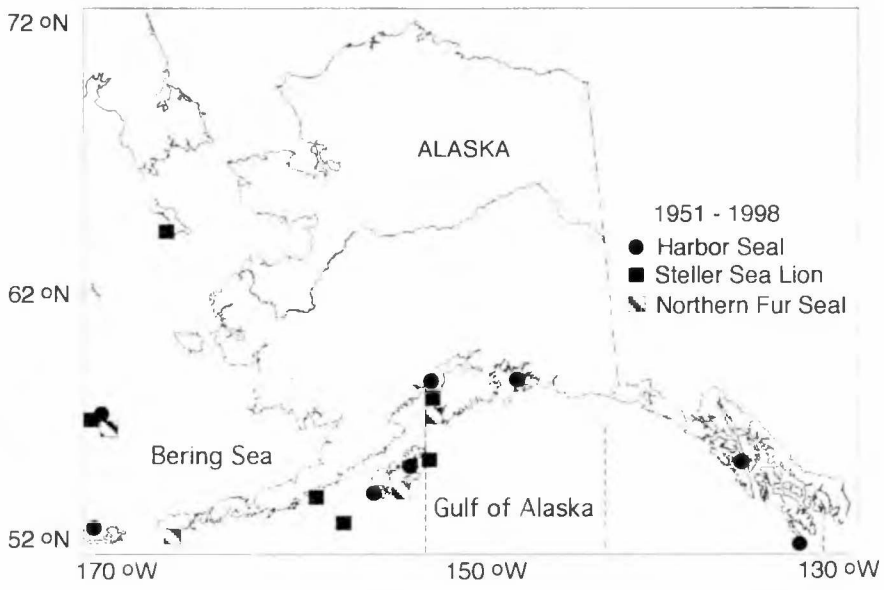

Figure 5.1. Collection locations of bone samples from Steller sea lions, northern fur seals and harbor seals, 1951-1998.

and at a maximum, six samples were available. Overall, an average of two specimens was available per year for all species.

Collagen extraction

Bone samples were well preserved and free of humus and tissues. Collagen was extracted following the procedure described in detail in Matheus (1997). Approximately 1 gm of bone was either cut as a solid piece or shaved from the mandible or the shaft of a long bone. Only cancellous bone was used for extraction due to the larger quantity of collagen it contains. The bone samples had lipids removed by a methanol/chloroform procedure described in Bligh and Dyer (1959) prior to demineralization. The bone was allowed to demineralize in 1N HCl for approximately seven days at 5°C; fresh acid was added to the samples every day. The remaining material was rinsed and then boiled in deionized water for approximately eight hours to dissolve the collagen and precipitate peptides. The solution was passed through a 0.45μ filter, and the filtrate was dried in an aluminum dish at 60°C for a minimum of 48 hours.

Mass spectrometry

Subsamples of each tissue (1-1.5 mg) were combusted and analyzed for stable isotope ratios with a Europa 20/20 continuous flow isotope ratio mass spectrometer. All samples were analyzed in duplicate. Stable isotope ratios were expressed in the following standard notation:

$$\delta X\ (‰) = (R_{sample} / R_{standard} - 1) \times 1000$$

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively. $R_{standard}$ for ^{13}C is Pee Dee Belemnite; for ^{15}N it is atmospheric N_2 (air). If the difference between replicates was greater than 0.5‰, samples were re-analyzed. Analytical error for samples was approximately ± 0.2‰ for both carbon and nitrogen.

RESULTS

Nitrogen isotope values for harbor seals ranged from 14.0 to 20.5‰ with a mean of 17.2 ± 1.6‰. The $\delta^{15}N$ for northern fur seals ranged from 15.2 to 20.1‰ with a mean of 17.2 ± 1.5‰ and the $\delta^{15}N$ for Steller sea lions ranged from 16.2 to 21.9‰ with a mean of 18.5 ± 1.4‰. Harbor seal collagen $\delta^{13}C$ values ranged from -12.0 to -16.4‰ with a mean of -14.2 ± 1.0‰. The $\delta^{13}C$ of collagen from northern fur seals ranged from -13.0 to -16.7‰ with a mean of -14.4 ± 1.1‰ and the Steller sea lion collagen $\delta^{13}C$ ranged from -12.5 to -15.8‰ with a mean of -14.3 ± 1.0‰ (Table 5.1). The between-animal and interannual variability in the $\delta^{13}C$ of all three species ranged from less than 1‰ to as much as 5‰.

Analysis of joint distribution in the $\delta^{13}C$ and $\delta^{15}N$ revealed differences among the three pinniped species (MANOVA: Wilks Lambda $F_{4, 130} = 3.30$, $P = 0.013$). Two separate analysis of variance tests (ANOVA) were then conducted to determine if nitrogen or carbon isotopes differed amongst the species. Univariate tests revealed that the three species segregated isotopically for only $\delta^{15}N$ ($P = 0.002$). Bonferroni

Table 5.1. Mean stable isotope ratios of bone collagen from harbor seals, northern fur seals and Steller sea lions. SEGOA = southeastern Gulf of Alaska, CGOA = central Gulf of Alaska, WGOA = western Gulf of Alaska, BS = Bering Sea.

Species	n	Year	Location	Mean $\delta^{13}C$	Mean $\delta^{15}N$
Harbor seal	2	1951	CGOA	-13.9 ± 0.5	20.2 ± 0.0
	1	1952	CGOA	-13.6	17.7
	1	1952	WGOA	-14.6	14.7
	1	1953	BS	-14.4	18.9
	1	1954	WGOA	-14.6	18.4
	2	1955	CGOA	-14.8 ± 0.2	17.9 ± 1.4
	2	1956	CGOA	-14.8 ± 0.8	17.3 ± 0.2
	1	1962	CGOA	-14.9	17.2
	2	1964	WGOA	-13.9 ± 0.9	16.1 ± 0.2
	5	1965	SEGOA	-13.2 ± 0.7	16.4 ± 0.7
	1	1965	WGOA	-13.1	16.1
	1	1966	CGOA	-13.4	16.9
	1	1966	WGOA	-14.8	17.7
	1	1966	BS	-14.2	19.4
	2	1968	BS	-14.3 ± 2.1	14.8 ± 0.8
	1	1969	BS	-15.4	14.4
	1	1970	WGOA	-12.0	16.9
	1	1970	BS	-13.6	19.5
	1	1971	WGOA	-13.2	17.4
	1	1971	BS	-14.4	16.9
	1	1972	CGOA	-13.6	20.1
	2	1972	BS	-13.7 ± 0.2	16.5 ± 0.2
	1	1973	CGOA	-14.3	16.4
	1	1973	BS	-15.6	20.5
	1	1974	BS	-14.4	16.5
	3	1975	CGOA	-14.7 ± 1.2	16.4 ± 1.2
	1	1976	WGOA	-13.7	17.0
	2	1977	WGOA	-14.7 ± 0.3	16.4 ± 0.5
	2	1978	CGOA	-13.5 ± 0.8	16.9 ± 0.3
	1	1978	WGOA	-14.4	18.4
	2	1979	BS	-14.1 ± 0.5	18.4 ± 0.6
	2	1980	WGOA	-15.6 ± 0.5	16.3 ± 0.2
	1	1981	CGOA	-12.2	15.6
	1	1981	BS	-13.5	20.0
	2	1985	WGOA	-14.8 ± 0.6	18.9 ± 1.5

Table 1. - cont.

Harbor seals	1	1989	CGOA	-14.0	18.7
	1	1993	CGOA	-15.8	17.2
	2	1995	SEGOA	-15.0 ± 0.6	19.0 ± 1.4
	1	1995	BS	-13.4	15.6
	1	1996	SEGOA	-14.4	15.8
	5	1996	CGOA	-15.0 ± 0.9	16.2 ± 0.6
Northern fur seals	2	1952	BS	-13.2 ± 0.2	16.1 ± 0.8
	1	1954	WGOA	-13.7	16.9
	1	1955	BS	-15.2	16.3
	1	1957	CGOA	-13.1	20.1
	1	1957	WGOA	-13.7	19.9
	1	1960	BS	-15.2	16.5
	1	1961	CGOA	-16.7	16.7
	1	1961	BS	-15.2	16.8
	1	1965	BS	-15.2	15.5
	1	1976	WGOA	-13.8	18.4
	1	1976	BS	-13.8	16.8
	1	1995	BS	-15.3	17.2
Steller sea lions	2	1953	BS	-14.1 ± 0.6	20.3 ± 0.9
	3	1956	CGOA	-13.0 ± 0.3	18.0 ± 0.6
	1	1957	CGOA	-14.5	18.5
	1	1958	BS	-12.5	17.0
	2	1960	WGOA	-12.8 ± 0.1	18.5 ± 0.4
	1	1960	BS	-14.2	16.4
	1	1961	BS	-13.2	21.9
	1	1965	BS	-14.6	18.8
	1	1966	CGOA	-13.1	20.4
	1	1969	CGOA	-14.3	18.7
	2	1971	BS	-14.9 ± 0.3	18.9 ± 1.6
	1	1974	BS	-12.9	20.0
	1	1977	BS	-14.9	18.5
	1	1978	WGOA	-15.2	17.5
	1	1979	BS	-15.0	17.0
	1	1988	CGOA	-14.6	18.5
	1	1989	WGOA	-15.6	17.0
	2	1993	BS	-15.7 ± 0.1	18.1 ± 1.9
	2	1994	BS	-14.9 ± 0.1	18.0 ± 0.1
	3	1995	BS	-14.2 ± 0.2	18.7 ± 0.8
	1	1996	BS	-15.4	17.0
	1	1997	WGOA	-15.8	17.8

correction tests for $\delta^{15}N$ showed that Steller sea lions had higher mean nitrogen isotope ratios than harbor seals and northern fur seals ($P = 0.015$ and $P = 0.014$, respectively). No differences in either isotope were detected among the defined regions for any of the three species (MANOVA: Wilks Lambda $F_{6, 130} = 0.58$, $P = 0.750$).

Analysis of joint distribution in the $\delta^{13}C$ and $\delta^{15}N$ for all years revealed that only $\delta^{13}C$ differed significantly during the forty-seven year period (MANOVA: Wilks Lambda $F_{70,130} = 1.558$, $P = 0.015$). When two separate analysis of variance tests (ANOVA) were conducted for each isotope, Steller sea lions had a significant decline in $\delta^{13}C$ (Kruskal-Wallis $P = 0.004$). The declining trends in harbor seal and northern fur seal $\delta^{13}C$ was not statistically significant by this method ($P = 0.298$ and $P = 0.072$, respectively). Separate regressions of nitrogen and carbon isotope ratios were conducted against year for the combined and individual species. Regression analysis of $\delta^{15}N$ against year showed no significant relationship during the forty-seven year period either in combined or individual species (Figure 5.2). Regression analysis of $\delta^{13}C$ showed a significant decline in the Steller sea lions ($P < 0.001$) (Figure 5.3) and a decrease, although not statistically significant at the $P = 0.05$ level, in both the harbor seals and northern fur seals ($P = 0.108$ and $P = 0.375$, respectively) (Figure 5.4 and 5.5). The sea lion $\delta^{13}C$ declined an average of 1.9‰ from 1953 through 1997. The lack of fur seal samples in recent years hinders the detection of any trends.

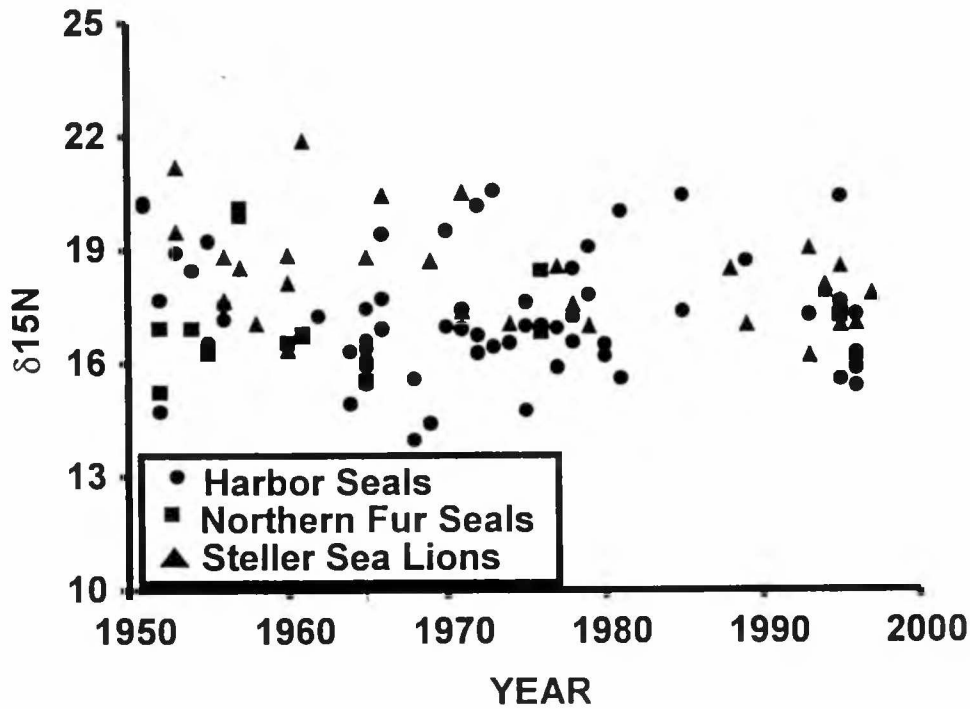

Figure 5.2. $\delta^{15}N$ values of bone collagen for Steller sea lions, northern fur seals and harbor seals from the Bering Sea and Gulf of Alaska, 1951-1997.

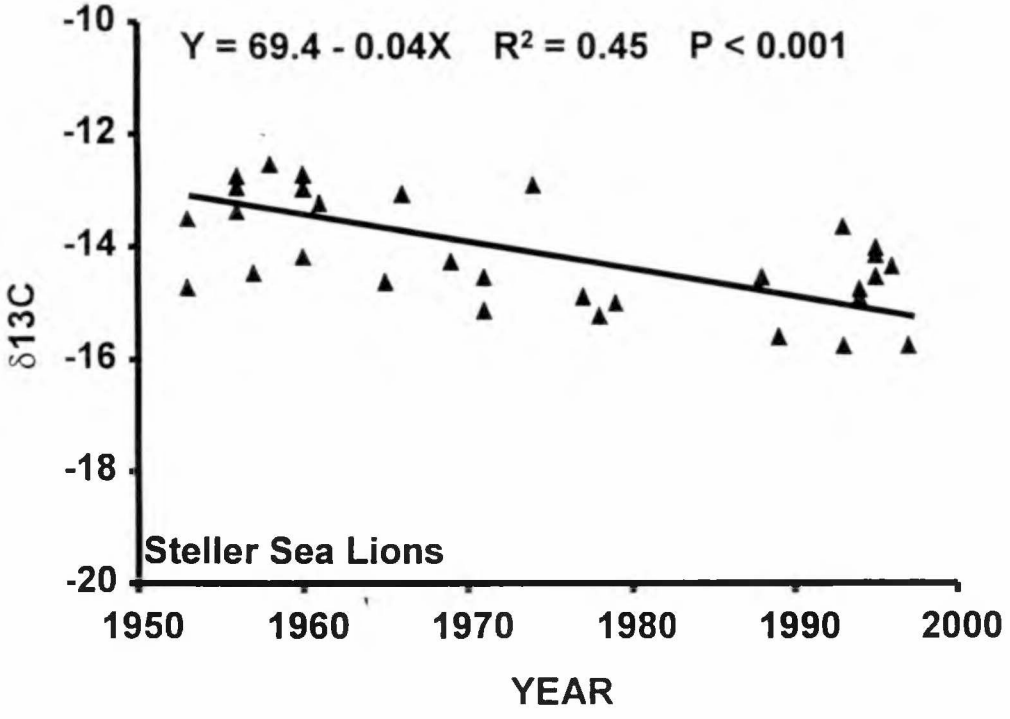

Figure 5.3. $\delta^{13}C$ values of bone collagen for Steller sea lions for the Bering Sea and Gulf of Alaska, 1953-1997.

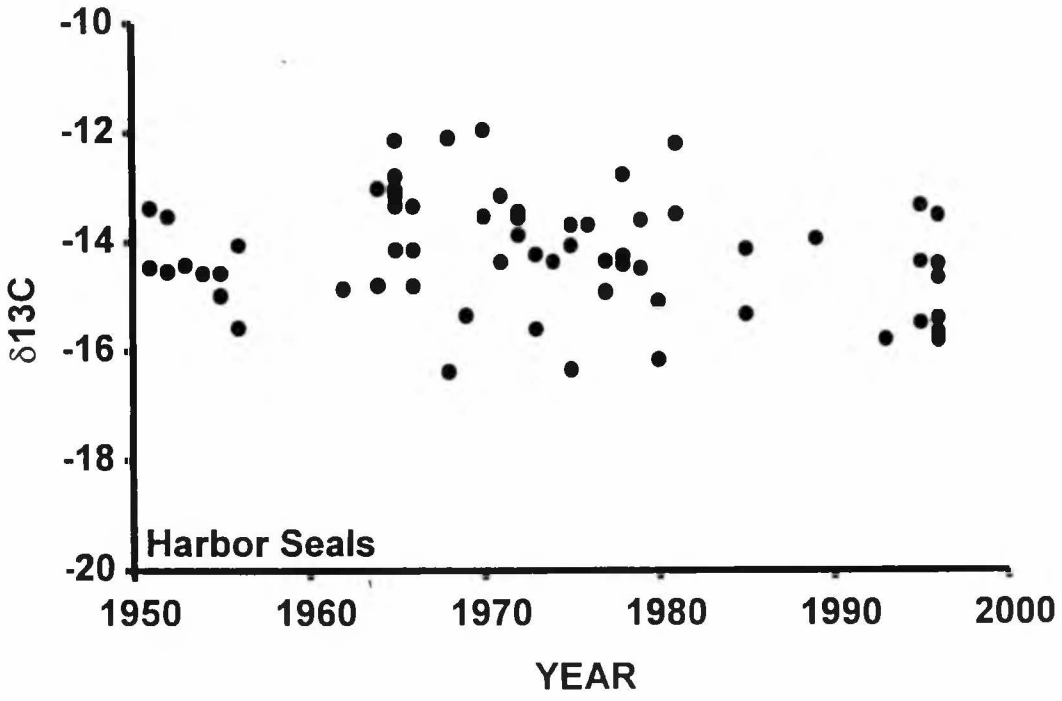

Figure 5.4. $\delta^{13}C$ values of bone collagen for harbor seals from the Bering Sea and Gulf of Alaska, 1951-1996.

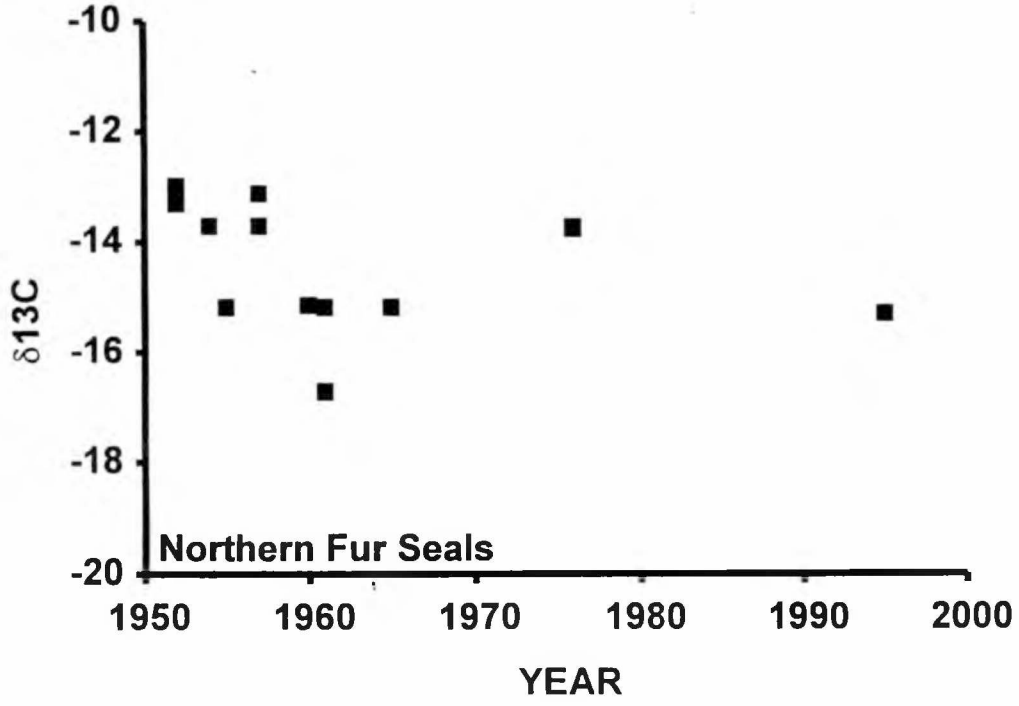

Figure 5.5. $\delta^{13}C$ values of bone collagen for northern fur seals from the Bering Sea and Gulf of Alaska, 1952-1995.

DISCUSSION

The major features of the data suggest there have been no long-term changes in $\delta^{15}N$ over the approximately 47-year period for all three species. A significant decline in $\delta^{13}C$ of Steller sea lions occurred during this period. This decline is also weakly suggested in data from the two species of seals. The trends, or lack thereof, appear to be uniform across regions. Three processes that may account for the changes in stable isotope ratios include temporal changes in diet/trophic position, foraging habits/location and isotopic composition of the base of the food web.

Diet/trophic variability

The present day diets of Steller sea lions, northern fur seals and harbor seals consist of a number of similar prey species but the composition may differ according to preferential prey and locally available species. Harbor seal diets appear to consist of mostly pelagic and semi-demersal fishes and benthic invertebrates, including herring, juvenile pollock and octopus (Pitcher 1980). The diet of northern fur seals appears to be largely composed of squid and juvenile pollock while Steller sea lions forage heavily on larger adult pollock, Pacific cod and flatfishes (Kajimura 1985; Sinclair et al. 1997; this study).

The $\delta^{15}N$ values obtained from the pinniped collagen suggest that Steller sea lions may feed at a slightly higher trophic level than the harbor seals and northern fur seals. Hobson et al. (1997) found similar results for Steller sea lions and northern fur seals from the Gulf of Alaska and concluded that the sea lions were consuming more

large-size pollock, which were enriched in $\delta^{15}N$ relative to the juvenile pollock and squid that the fur seals were predominantly relying on. Perez and Bigg (1986) noted that northern fur seals in the eastern Aleutians and Gulf of Alaska between 1958 and 1974 fed largely on sandlance, capelin and herring. The diets of both the forage fish and juvenile pollock consist primarily of zooplankton and thus are at a similar trophic position consistent with similar $\delta^{15}N$ values. Pitcher (1980) noted that walleye pollock was the predominant prey in both the Steller sea lion and harbor seal diets in the Gulf of Alaska during the mid-1970s, but that each species foraged on different sizes of pollock. Steller sea lions were eating pollock significantly larger than those eaten by harbor seals. These larger pollock are mainly piscivorous, often feeding on smaller pollock and forage fish, whereas the smaller pollock feed largely on zooplankton and juvenile forage fish. These trophic differences result in more enriched nitrogen isotope values in the large pollock by ~2.5 ‰ (DeNiro and Epstein 1981; this study) and would result in higher $\delta^{15}N$ in the sea lions.

Changes in prey composition during the 1970s, as previously described, may have altered the isotope ratios in the pinnipeds. Adult and some juvenile pollock, which are currently predominant in the diets of many of these animals, generally have more enriched $\delta^{13}C$ and $\delta^{15}N$ values than the once prevalent clupeid fishes (herring, capelin) because they largely forage at a higher trophic level than the clupeids (Hobson et al. 1997; Merrick et al. 1997; this study). If these pinnipeds shifted to forage at a higher trophic level, we would expect increases in both the nitrogen and carbon

isotope ratios (DeNiro and Epstein 1978, 1981; Rau et al. 1983). However, fish having high lipid content, i.e., clupeids, generally have more depleted ^{13}C than a fish naturally low in lipids, i.e., pollock and cod as well as being depleted due to the trophic level difference. Only ~3‰, representing a trophic level change, was evident in ^{15}N with no corresponding change due to lipids (Tieszen et al. 1983; Hirons unpubl. data). Therefore, the lack of co-variance between carbon and nitrogen isotopes does not support a prey-switching scenario for these pinnipeds.

Foraging habits/location

Harbor seals have strong site affinity and feed in nearby coastal locations in Bristol Bay in the Bering Sea and throughout the northeastern Pacific Ocean (Pitcher 1980; Frost et al. 1999; this study). Northern fur seals forage in the shelf break and offshore waters of the Bering Sea, the Gulf of Alaska and as far south as California during their annual migration to and from the Pribilof Islands in the Bering Sea (Kajimura 1984; Kajimura 1985; Goebel et al. 1991; Loughlin 1993; Sinclair et al. 1994; Merrick 1995; NRC 1996). Steller sea lions spend their year foraging predominantly over the continental shelf and in offshore waters in the southern Bering Sea and Gulf of Alaska while traveling among rookeries and haul-out sites (Kenyon and Rice 1961; Merrick et al. 1997). Although the movements and life histories of our individual pinnipeds are unknown, and only generally known for each species, we assume the $\delta^{13}C$ values in the bone collagen represent prey from the northeastern Pacific and Bering Sea.

As discussed, these pinnipeds have heterogeneous diets that often include prey from different trophic levels and regions. Schell et al. (1998) noted the existence of isotopic gradients in the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton in eleven subregions from the Bering Sea and, recently, similar isotopic gradients were identified in the continental shelf waters of the Gulf of Alaska using the same taxa of zooplankton (this study). Regions of high primary productivity, including the shelf break in the Bering Sea and continental shelf in the Gulf of Alaska, contain zooplankton with higher $\delta^{13}C$ and $\delta^{15}N$ than areas further offshore. Thus variations in the year-to-year movement patterns of both pinnipeds and prey, as well as differences in prey availability, may cause fluctuations in the isotopic ratios among individual pinnipeds.

Pinnipeds may vary forage locations as they move to and from rookeries seasonally or as they follow potential prey and, all the while, the bone collagen is integrating these complex movements. The isotope ratios in harbor seals, which tend to have a strong site affinity, would represent the prey items in the seals' coastal feeding locations (Pitcher 1980). Such observations may help explain the high between-animal isotopic variability. The nitrogen isotopes in the Steller sea lions likely reflect the prey often found in near- and offshore waters in the Bering Sea and in the Gulf of Alaska, whereas the isotopic ratios in northern fur seals could reflect prey consumed in the Bering Sea, the Gulf of Alaska and as far south as the offshore waters of California as they migrate (Goebel et al. 1991; Loughlin 1993; Merrick 1995). Isotope data could reveal long-term changes in feeding location if these locations were

isotopically distinctive in terms of their prey. If the zooplankton data (Schell et al. 1998; this study) are indicative of regional patterns of changes in prey isotope composition, changes in foraging location should result in changes in both the carbon and nitrogen isotopes in the pinniped species examined. Thus, our data are not consistent with major changes in feeding location as the sole mechanism explaining these trends. The Steller sea lion data could be explained by a shift to offshore feeding (lowering $\delta^{13}C$) accompanied by a shift to a higher trophic level prey, i.e., offshore adult pollock which may have the same $\delta^{15}N$ as lower trophic level, inshore capelin (this study). The isotope ratios of the higher trophic level pollock would be offset by the lighter isotope ratios at the base of food webs in pelagic waters.

A decrease in $\delta^{13}C$ in consumers can be due to a diet switch from isotopically enriched, benthic organisms to a more isotopically depleted, pelagic diet. Benthic organisms tend to be more enriched in ^{13}C due to an increase in the length of the food web by microorganisms, although no subsequent enrichment in ^{15}N was reported (Hobson and Welch 1992; France and Peters 1997). No historical or current data provides evidence of this happening with sea lions or fur seals but harbor seals currently eat a mix of pelagic and benthic prey (this study). In summary, our data do not support the hypothesis of significant changes in trophic level of prey organisms for these mammals, unless such effects were masked by simultaneous changes in factors such as feeding location or prey isotopic composition.

Environmental influences

A third process that could account for the observed trends is one in which the stable isotope composition at the base of the food web changes over time. Based on the Steller sea lion data, such a mechanism would require coherent trends over a large region spanning the Gulf of Alaska and Bering Sea. Further, such changes should be registered in much of the food web in this region. The declining trends in the harbor seals and northern fur seals are consistent with this hypothesis, though are less significant than the sea lions. Steller sea lions were the only one of the three pinniped species to show significant long-term declines in their carbon isotope ratios. This may be due to the spatial variations in migration and habitat between the sea lions and the other two species. Harbor seals forage in productive coastal waters enriched in ^{13}C while fur seals and sea lions often forage in less productive, offshore waters where the $\delta^{13}C$ values are more depleted (Schell et al. 1998; this study). The heterogeneous diets and localized habitats of harbor seals may have contributed to large inter-animal variability hindering statistical detection. The available northern fur seal samples were too few to vigorously confirm this hypothesis. We conclude that our data at present cannot rule out isotopic variation due to forage location as a potential explanation.

The $\delta^{13}C$ values in top trophic level marine organisms can be changed without modifying the animals' trophic position, as this value is set by the composition of phytoplankton in the food web. The isotopic composition of the phytoplankton is affected by the isotopic composition of dissolved inorganic carbon and the

fractionation that occurs during the growth of plant cells. These isotopic differences are incorporated into all consumers in the food web.

Anthropogenic CO_2 from the burning of fossil fuel, which is depleted in ^{13}C, has resulted in a decrease in $\delta^{13}C$ of oceanic dissolved inorganic carbon (DIC), commonly referred to as the "oceanic Suess effect" (Kroopnick 1985). The degree of $\delta^{13}C$ change from the Suess effect varies spatially in the ocean due to processes such as ocean circulation and mixing. Smaller decreases have been observed in subpolar oceans ($< -0.15‰$ decade^{-1}). The high latitude zone of the Pacific Ocean, where these seals and sea lions reside, is subject to upwelling and deep water mixing in the winter. These events tend to reduce the Suess effect due to dilution from "old", deep water and the limited time for CO_2 equilibration to take place (Quay et al. 1992; Gruber et al. 1999, Sonnerup et al. 1999). Given the time period of this study, the Suess effect would account for a depletion of no more than 0.75‰ in $\delta^{13}C$ over five decades. The $\delta^{13}C$ decrease observed in the Steller sea lion bone collagen is too large of a different temporal pattern to be explained simply by the increase in anthropogenic carbon during the past five decades.

Rapid use of CO_2 during photosynthesis can lead to an increase in the $\delta^{13}C$ of the plant cells if the rate of CO_2 replenishment is slower than usage (Goericke et al. 1994). Laws et al. (1995) and Popp et al. (1998) have empirically demonstrated strong, positive relationships between the $\delta^{13}C$ of a marine diatom and cell growth rates while Bidigare et al. (1997) showed a similar correlation among carbon isotope

values and empirical haptophyte algal cell (coccolithophore) growth rate in natural marine settings. According to this process, the declining trends in bone collagen $\delta^{13}C$ imply a long-term decline in phytoplankton cell growth rates, and hence primary productivity, over this period.

The productivity hypothesis assumes that other factors controlling phytoplankton $\delta^{13}C$ ratios, such as $[CO_2]aq$ and phytoplankton cell size (Rau et al. 1989), are relatively constant over this period. The offshore subarctic Pacific is dominated by small-sized phytoplankton whose populations stay relatively constant throughout the year (Martin et al. 1989). Phytoplankton growth is limited by photosynthetically active radiation (PAR) and nutrient availability though the subarctic Pacific Ocean is not nitrogen-limited. Studies conducted by Miller et al. (1991) in the subarctic Pacific indicate the phytoplankton are dominated most of the year by small flagellates and that larger phytoplankton, particularly large diatoms, may be limited by iron availability. Iron input from land is proposed as being the reason large diatoms dominate over the continental shelf region during a portion of the year (Martin and Fitzwater 1988; Boyd et al. 1996; Martin et al. 1989). There is insufficient data to evaluate long-term changes in the phytoplankton community in this region and any subsequent effects on ecosystem $\delta^{13}C$. A seemingly uncommon occurrence of coccolithophorid blooms has occurred in the Bering Sea since 1997. These blooms are known to occur when a highly stratified water column in warm, calm seas hampers

nutrient cycling (Sukhanova and Flint 1998). However, this change is too recent to be strongly represented by our bone samples.

Physical factors affecting changes in wind intensity and mixed depth layers in the northeast Pacific appear to have affected productivity in the region. Polovina et al. (1995) observed a shallowing of the winter mixed layer depth in the subarctic North Pacific from 1977-1988 and attributed the change to an intensification of the Aleutian Low Pressure System. Recent data presented by Freeland et al. (1997) and Mackas et al. (1998) for Station Papa (50°N 145°W) indicate that summer nitrate concentrations and mixed layer depths have decreased. The model used by Polovina et al. (1995) to test effects of changing mixed layer depths predicted potentially large changes in phytoplankton production and zooplankton stock in the northeastern Pacific.

Since 1976, northeastern Pacific and southern Bering Sea water temperatures have increased and the extent of sea ice cover in the Bering Sea has diminished (Niebauer 1988; Royer 1989). The timing of this change in thermal structure corresponds to shifts in species composition of fish and invertebrates (Ebbesmeyer et al. 1991; Anderson et al. 1997; Anderson and Piatt 1999). In the Gulf of Alaska, there has been a long-term trend of decreasing salinity due to increased runoff. The warmer temperatures and lower salinity would increase stability of the water column and that could impede the supply of nutrients to the surface waters in some environments. Data from the Bering Sea PROBES study demonstrated decreased primary production during times of the year when wind mixing diminished (Walsh and McRoy 1986).

However, increased water column stability has been suggested as being favorable for phytoplankton growth in some settings (Gargett 1997).

Data on chlorophyll and zooplankton biomass may provide some evidence of past productivity fluctuations. Sugimoto and Tadokoro (1997) compiled estimates of zooplankton biomass and chlorophyll concentrations for the eastern Bering Sea spanning the years 1954-1994. These authors compiled a more limited data set for both variables for the northeastern Pacific Ocean. Values for both regions showed increases in both phytoplankton and zooplankton standing stocks during the mid-1960s and a general decline after that point. Carbon isotope values for organic matter in a sediment core from Skan Bay, Unalaska, in the Aleutian Islands of Alaska, revealed a decline of almost 1.5‰ between 1950 and 1998 (Finney unpubl. data).

The decline in the $\delta^{13}C$ of Steller sea lion bone collagen is similar to the pattern that Schell (2000) observed in bowhead whale baleen from the Bering Sea over the same period of time, 1947 to 1998. These baleen whales feed heavily on zooplankton stocks that have integrated the previous season's primary production and now serve as a proxy for the average annual productivity. The average carbon isotope ratios in bowhead whale baleen laid down in the Bering and Chukchi seas were used to estimate the relative interannual changes in primary production in the Bering Shelf ecosystem using relationships from Laws et al. (1995) and Bidigare et al. (1997). Assuming the correlation between the measured haptophyte algae growth rates and changes in $\delta^{13}C$ are similar to phytoplankton growth in the Bering Sea, the isotope

ratios in baleen imply a decline of 30-40% in ecosystem productivity between 1966 and 1997.

Abundant evidence exists for environmental changes in the North Pacific Ocean and the Bering Sea in recent decades. However, changes within subregions may not always coincide with one another. The hypothesis that changes in primary productivity were coherent over this large region, a bottom-up mechanism, has been proposed by numerous researchers to explain changes in abundances of many different organisms in this region (Beamish and Boullion 1993; Francis and Hare 1994; Hare and Mantua 2000). Oceanographic and biological differences can result in a lack of concordance that has been illustrated by differences in salmon abundances, for example. Large increases in salmon catch in the Gulf of Alaska and Bristol Bay (southeast Bering Sea) occurred during this period. However, the salmon data is often interpreted as increasing productivity (Francis and Hare 1994), in contrast to the carbon isotope data.

A variety of physical and biological indices have provided evidence of rapid changes known as regime shifts around 1976 and 1989 (e.g. Ebbesmeyer et al. 1991; Brodeur and Ware 1992; Francis and Hare 1994; Polovina et al. 1995; Anderson et al. 1997; Beamish et al. 1997; Anderson and Piatt 1999; Hare and Mantua 2000). Evidence of declines in Steller sea lions, northern fur seals and harbor seals since the 1970s (Merrick et al. 1987; Pitcher 1990; Alaska Sea Grant 1993; Merrick et al. 1997) has prompted some researchers to try and link the declines to the reported regime

shifts. As bone collagen has a relatively slow turnover rate that may be greater than 10 years, the isotope ratios present in the collagen would be an integrator of that period of their lifetime so that abrupt changes would be hard to detect.

Summary

An overwhelming amount of evidence suggests that the environment of the North Pacific Ocean and Bering Sea has changed during the past several decades. Furthermore, the changes in the physical environment may be associated with changes in the primary production in the region. The Committee on the Bering Sea Ecosystem assessed the likelihood of various potential causes on the declines of these three pinniped species and found that climate effects and environmental changes were likely factors affecting the fish community and food availability for these animals (NRC 1996). Reduction in food, which subsequently leads to population declines if depletion is great enough, seems to be supported for Steller sea lions (Trites 1992; Merrick 1995). Our $\delta^{15}N$ data show no major shift in trophic status for any of the three pinniped species during the past five decades even though changes in prey composition and feeding locations have likely occurred.

Temporal changes in the diets of these pinnipeds may have contributed to changes in the animals' isotope ratios but detection of these changes can be complicated by the ecology of each of these animals. Simultaneous changes in foraging locations, e.g. nearshore vs. offshore, as well as foraging habits, e.g. switching from a benthic to pelagic diet, could alter the isotopic composition of the sea lions.

Heterogeneity in diet and forage locations for these pinnipeds would likely prevent either of these mechanisms from completely explaining the decline in the carbon isotope ratios. Extensive changes in the physical environment have occurred in the northeastern Pacific Ocean and Bering Sea for decades but their impact on primary productivity can only be surmised. Our understanding of how pinnipeds are impacted by these events is impeded by the absence of detailed or adequate long-term monitoring of primary productivity in these regions. However, carbon isotope ratios may provide a means to measure the impact of environmental change in upper trophic level organisms.

Marine mammal populations can be expected to change with time in response to environmental perturbations. The large-scale declines seen in the Bering Sea and Gulf of Alaska pinnipeds are unusual because they appear to have happened in a short time whereas some populations of the same species have remained stable or increased in other areas of the North Pacific. Short-term environmental changes, such as El Niño events, would have only a limited impact on these pinniped populations by reducing food availability (Trillmich and Ono 1991). Short-term changes that could alter the carbon isotope ratios in the marine food webs would likely be tempered in the bone collagen records due to the relatively slow turnover rate of isotopes in this tissue. The magnitude of changes observed in the North Pacific seem to warrant further investigation on their impact to marine mammal populations, and the use of isotope ratios should enhance our understanding of these changes.

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CHAPTER 6

CONCLUSIONS

Pinniped populations have been decreasing in the Bering Sea and Gulf of Alaska for at least three decades. The goal of this dissertation has been to examine the trophic dynamics of Steller sea lions, northern fur seals and harbor seals in these regions to determine if dietary changes have contributed to these declines. Stable carbon and nitrogen isotope ratios were used as an indicator of trophic level in these animals and their potential prey species. A transfer of carbon and nitrogen isotope ratios from the diet to the pinniped tissues, with associated fractionation, allows food webs to be traced. The stable isotope data for each of these species resulted from a complicated mix of prey organisms, geographic isotope gradients and environmental conditions.

Vibrissae (whiskers) from the pinnipeds are keratin-based structures that grow and are replaced throughout their lifetime and provide a timeline of trophic information that other tissues cannot. Due to metabolic fractionation differences, isotope ratios from the vibrissae could not be directly compared with those from prey muscle tissue. A comparison of muscle and vibrissae from the same animals in all three species revealed that keratin was enriched in $\delta^{13}C$ over muscle by a consistent amount while $\delta^{15}N$ deviated only slightly. Stable isotope values in the vibrissae were

normalized to muscle to adjust for these fractionation differences and this allowed for a comparison of vibrissae isotope ratios to those from prey muscle samples.

Growth rates of the vibrissae were studied in harbor seals and Steller sea lions from Alaska to determine what time period the isotope ratios represented. Information from both captive and wild harbor seals indicated that their vibrissae were grown for several months a year and then replaced annually with new whiskers. In contrast, Steller sea lions in captivity and in the wild had a much different growth pattern. Their vibrissae appeared to grow continuously and were maintained for several years. If either species were to break or damage a whisker, a new one was rapidly grown to replace it. The information gathered for the harbor seals was in sharp contrast to the hypothesis that vibrissae represented several years' growth, while the sea lion vibrissae grew as predicted. The growth rate experiment could not be conducted for northern fur seals at that time; however, similarities in carbon and nitrogen isotope ratio fluctuations between the fur seals and the sea lions, believed to result from geographic isotope gradients, imply a similar growth rate pattern exists in northern fur seals as in Steller sea lions.

The Pribilof Islands in the Bering Sea and the western portion of the Gulf of Alaska were the regions where the declines of northern fur seals and Steller sea lions, respectively, were first noted. The fluctuations in the carbon and nitrogen isotope ratios were highly correlated with each other in the vibrissae of both species and clearly had a repetitive, cyclic pattern. The fluctuations in both $\delta^{13}C$ and $\delta^{15}N$ of the

northern fur seals appeared greater than a trophic step alone, i.e., greater than 1‰ and 3‰, respectively. When those values were compared with probable prey items throughout the northeastern Pacific Ocean, stable isotope ratios from northern fur seal vibrissae were consistent with a diet of predominantly squid and juvenile walleye pollock. Steller sea lions had somewhat modified oscillations in their $\delta^{13}C$ and $\delta^{15}N$, but those shifts also corresponded to a diet of gadids (both Pacific cod and walleye pollock) and some Atka mackeral from the Bering Sea and Gulf of Alaska. The fluctuations in the stable isotope ratios in the vibrissae of both the sea lions and fur seals are consistent with the hypothesis that they provide information not only on trophic level but also on geographic movement.

Some adult and subadult harbor seals in Prince William Sound expressed isotopic fluctuations in their vibrissae that suggested the seals were feeding at times within the sound and at other times in the Gulf of Alaska. The isotope ratios of prey were considerably lower in the gulf than within the sound, and the more negative ^{13}C values represented in the seal vibrissae indicate that these prey were likely consumed by the seals. The most positive ^{13}C values indicated benthic prey were likely a large component of the seals' diet. These data supported the hypothesis that prey outside Prince William Sound were isotopically depleted and were being foraged on by some seals. Whether the observed isotopic fluctuations in the seal vibrissae were entirely the result of geographic differences in food webs needs to be determined.

Interannual variability in the seals' movements (Frost et al. 1999) seems to provide some evidence of a changing pattern, in which seals forage within and outside the sound during some years and forage only within the sound in other years. Tagging data from 1992 showed seals remaining in the sound while subsequent years showed seals traveling, and presumably foraging, in Prince William Sound and the Gulf of Alaska. Vibrissae isotope data seem to be consistent with this hypothesis. In most cases vibrissae were collected before tracking data was obtained for the seals. There were no fluctuations in both isotope ratios in vibrissae from seals sampled in May 1993. These isotope ratios in the vibrissae would represent the prey isotope ratios for 1992-1993. Yet all subsequent years of the study showed large isotopic fluctuations in the vibrissae that appeared to correspond to prey found outside the sound. These data corresponded to those tagging data that also revealed seals traveling outside the sound.

Dietary changes in seals and sea lions, resulting from changing prey abundances since the mid 1970s, were expected to result in changes in trophic level and contribute to the declining pinniped populations. While pinniped tissues were being collected and analyzed throughout the 1990s, no obvious change in trophic level was observed. In an effort to look for changes during a continuous span of time, five decades of bone samples were analyzed for all three species. However, the $\delta^{15}N$ in the bone collagen also showed no evidence of trophic level change. A significant decline in the carbon isotope ratios of Steller sea lions during that time period

suggested that a decline in the primary production at the base of the food webs had occurred. The decline in $\delta^{13}C$ was not detected in northern fur seals presumably due to a lack of samples available after 1976. Likewise, no significant change in the carbon values in harbor seals was observed, presumably due to their localized habitats. These isotopic data, as well as those from Schell (2000), Sugimoto and Tadokoro (1997) and Finney (per. comm.), were consistent with a decline in primary productivity, and potentially carrying capacity, in the northeastern Pacific Ocean that may have resulted in diminished prey for the seals and sea lions.

Evidence of declining primary productivity from the $\delta^{13}C$ of sea lions' bone collagen indicates an impact on the populations has been occurring for at least fifty years, yet population declines have only been recorded for the past twenty-five to thirty years. Bone collagen used in this study is a decadal-long integrator of stable isotopes that would temper any rapid changes in the isotope record, which may have occurred closer in time to the noted pinniped declines. If decreased productivity is a factor in the pinniped declines, primary productivity may need to be suppressed for an unknown length of time before it impacts apex predators, like pinnipeds. It may also be possible that the productivity decline is not the sole cause of the declining pinniped populations but, rather, is acting in combination with other conditions. Primary productivity changes may have indirectly contributed to the declines by reducing or changing the animals' forage base. The changes in prey availability may have then resulted in the declining pinniped populations.

The isotopic data provide evidence that changes in the physical environment could be an underlying cause for the decline in these pinniped populations. An abundance of physical evidence already exists that indicates changes have occurred in our ocean climate. The linkage between ocean climate and biological production was supported by the associated increases and decreases in stocks of fish and shellfish at the time of the 1976-1977 regime shift (Anderson and Piatt 1999). Marine mammal and sea bird declines were noted at approximately the same time that clupeid and gadid biomasses were changing. Clearly, further research is needed in many areas besides pinniped biology to evaluate what impact environmental fluctuations have on organisms in the marine environment.

As with any research topic, particularly one dealing with marine mammals in the wild, pitfalls occurred during this study. Because many of the vibrissae were collected from live seals and sea lions and no one knew how many animals would be handled during a cruise, the number of vibrissae samples remained a virtually unknown quantity until sampling was completed. This study relied heavily on opportunistic sampling of not only pinniped tissues but also prey samples. Specific prey species were targeted for sampling and as many different geographic regions were sampled as possible, when ships of opportunity became available. The logistics of this study required multiple cruises and the assistance of many different people and agencies.

The growth rate experiment and vibrissae monitoring required adhering to set protocols that Mystic Aquarium and Vancouver Aquarium had with regards to their

captive pinnipeds. The experimental designs often had to be modified to fit the institutional criteria for when animals could be handled and which tissues could be sampled. Lapses in data collection and unannounced removal of subject animals forced delays in data acquisition. Both institutions were at such distances that only an annual visit could be managed. Some of these difficulties may have been avoided if direct contact with the subject animals could have been maintained.

Future research

The combined results from each of these studies have contributed to the body of knowledge on pinniped trophic status. Vibrissae have proven to be useful in providing trophic information in various temporal scales (e.g. weeks to months to years). The studies described here indicate that understanding the complex interactions between these pinnipeds and their marine environment is only just beginning.

As mass spectroscopy becomes more refined, smaller and smaller samples will be necessary for stable isotope analysis. The role of vibrissae will not only be how fast the vibrissae grow but how vibrissae are grown. Initial views of vibrissa segments from all three species with an electron microscope revealed that the inner core of a vibrissa appears to grow first and the external layers are added as growth ensues. This creates a conical shape within the vibrissae that may reveal even more detailed growth information, and thus when isotopes were incorporated, as finer resolution of vibrissa segmenting evolves. This growth pattern was not an issue

during this study as vibrissae segments used for analysis were much larger than the conical layers.

Additional growth rate studies on vibrissae need to be carried out on all of these species to further refine their growth period with respect to seasonality and age of the animals. It is also uncertain what effect fasting or other metabolic change has had on vibrissae growth. Similar, long-term growth rate studies on captive animals will be valuable in trying to answer these questions. Until effective recapture methods are available for wild pinnipeds, captive studies remain the best source for this type of information.

Geographic variability has been shown in this study, as well as Schell et al. (1998), to affect the isotope ratios of not only primary producers at the base of the food web, but the entire food web. Stable isotope ratios in vibrissae have not only provided trophic data on these animals but have also shown the potential in identifying locations where the seals and sea lions have fed. To increase their usefulness in identifying where an animal has eaten, the isotopic maps will need to be enlarged. The development of more detailed isotopic maps will require sampling zooplankton for isotopic analysis in more diverse locations throughout the expected ranges of these pinnipeds. Satellite- and radio-tagging of wild pinnipeds is being used more widely by researchers in their efforts to learn the secret habits of pinniped diving and traveling. Satellite tagging of selected seals and sea lions in the Gulf of Alaska are providing more precise geographic information but it can be cost prohibitive as the units are designed to remain with the animals only until the next

molting period, less than one year. Stable isotope ratios in vibrissae may prove to be a cost effective means of acquiring generalized feeding and location information for these elusive animals.

The greatest downfall most researchers have in their effort to identify the sources of the pinniped declines is the lack of any long-term data sets. Data on prey fish abundance in concert with pinniped population estimates cover little more than forty years at best. The types of declines being observed were not likely induced by short-term changes. Longer-term, retrospective studies will be needed to determine if the declining productivity, and subsequent carrying capacity, is a natural occurrence or anthropogenically produced. Bone and tooth collagen from pinnipeds continue to be the best source of tissue for stable isotope analysis. These types of tissues are available in museum collections for periods during the nineteenth and twentieth centuries. Samples prior to that time have been located in archaeological middens from sites around Alaska. Bones from potential prey species are also found in these sites and can be used to reconstruct pre-existing food webs. Long-term isotopic records regarding trophic and environmental changes are locked away in these tissues.

The field of stable isotope research is expanding with researchers employing lesser-known isotopes to help answer questions about biological and physical environments. Even with these advancements, stable isotopes alone will not provide the ultimate answer to the question of pinniped declines. Rarely does one technique answer an ecological question of this magnitude. The data stable isotopes provide, in

combination with other scientific techniques, are expected to contribute to the body of knowledge on pinniped ecology and help point the way to the answer regarding their declines.

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